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THE RENAL RESPONSE TO SUSTAINED ADMINISTRATION OF VASOPRESSIN AND WATER IN MAN*

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ABSTRACT

An evaluation of the effects of prolonged administration of vasopressin on water and electrolyte metabolism was made in 5 studies on 4 human subjects. In subjects receiving a high fluid intake, an initially rapid expansion of total body water associated with an excessive urinary excretion of sodium was observed. Ultimately, however, urinary osmolality declined, daily urinary output rose, and body water balance was re-attained. Concomitantly, excessive natriuresis and net loss of body sodium ceased, despite the presence of an expanded body fluid volume. Although administration of vasopressin and a high fluid intake was continued, a new steady state was achieved. This was characterized by persistent overhydration and hyponatremia. It is suggested that duration of the overhydrated state is a critical factor in determining the development of a reduced renal responsiveness to vasopressin. This response was never observed prior to the seventh day of vasopressin administration, and in two studies occurred when only slight overhydration was present. A clinically significant degree of sodium depletion was not produced by this procedure. The persistent hyponatremia was primarily the result of water retention. The present data provide certain criteria for the clinical evaluation of hyponatremic states in which sustained excessive secretion of antidiuretic hormone is suspected as the etiologic mechanism.

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THE role of vasopressin in the pathogenesis of clinical states of water retention remains undefined. It has been demonstrated that water retention induced acutely by administration of fluid and vasopressin is associated with marked natriuresis and the development of hyponatremia (1). This observation has suggested the possibility that certain clinical syndromes, characterized by water retention, excessive output of sodium in the urine and hyponatremia, might result from a sustained unregulated secretion of antidiuretic hormone (2–4). In order to assess this possibility more carefully, the present study was undertaken. The effects of relatively long-term administration of water and vasopressin on body water and electrolyte balances have been determined in human subjects.

METHODS AND PROCEDURE

All studies were conducted on a metabolic ward, where complete balances of sodium, potassium, chloride, nitrogen and water were measured. Diets, stools and urines were analyzed by methods previously outlined for this laboratory (5). In addition to the direct measurement of fluid intake and output, water balance was calculated by the method of Newburgh *et al.* (6). Osmolality of urine and serum was measured with a Fiske osmometer.

All subjects were given a constant diet. After a suitable control period, Pitressin® tannate in oil was administered in fractional doses at 12-hour intervals. Particular care was taken to shake the vials thoroughly, to insure complete and even suspension within the oil medium.

Subjects were selected from the wards or out-patient clinics of the Strong Memorial and Rochester Municipal Hospitals. None showed evidence of cardiac or renal disease. The following is a brief clinical summary of each subject in whom prolonged studies were performed:

E.C.: A 54-year-old man in whom studies were initiated after recovery from mild bronchopneumonia. He gave a history of periodic heavy alcoholic intake. There was slight hepatomegaly but no evidence of hepatic functional impairment. A liver biopsy performed one year after this study revealed no abnormalities.

L.P.: A 62-year-old man with known hepatic cirrhosis, manifested by edema and ascites in the past. At the time of study he was apparently well compensated and edema and ascites were absent despite a normal dietary salt intake.

H.W.: A 43-year-old man who had been hospitalized because of nonunion of an old femoral fracture. The patient was otherwise in good health.

R.L.: A 40-year-old man in whom metabolic studies were carried out after recovery from pneumonitis. He was in good general health without evidence of any chronic disease process.

RESULTS

Long-term administration of vasopressin to subjects receiving a high fluid intake

In 3 studies on 2 subjects, vasopressin was given in conjunction with a relatively high fluid intake for a period of seventeen to twenty-three days.

Figures 1 and 2 summarize the data from 2 separate studies on Subject

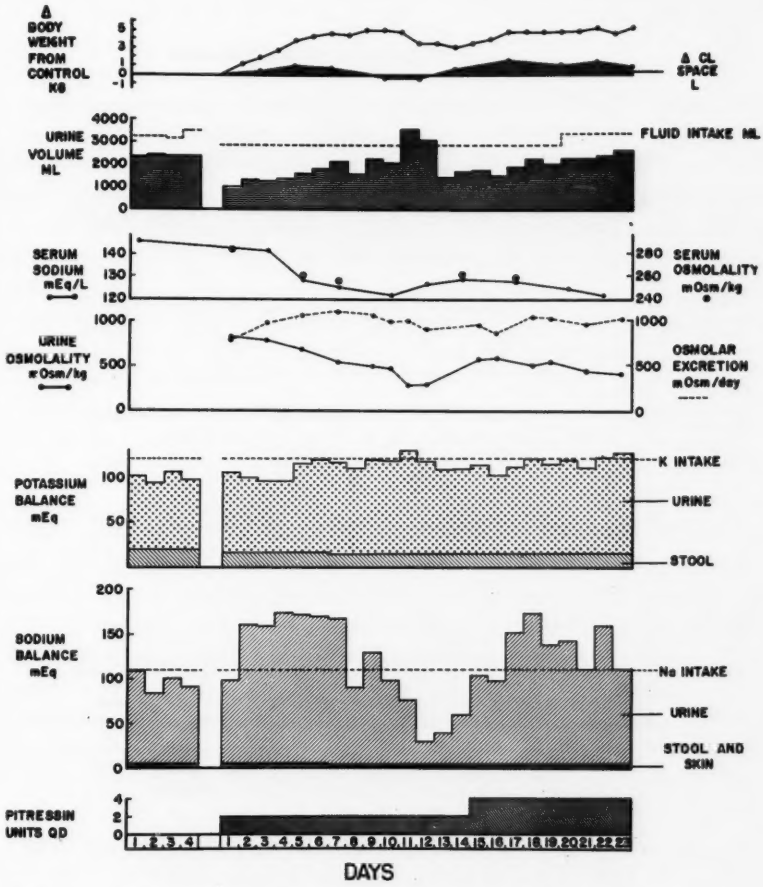

FIG. 1. Subject E.C. (Study #1). Effects of prolonged administration of vasopressin and a high water intake.

E.C. Vasopressin was given for twenty-three days in each. In the first study (Fig. 1), following a control period, daily administration of 2 units of Pitressin in oil and a constant fluid intake of 2800 ml. was initiated. The initial response, apparent during the first six days of vasopressin administration, consisted of progressive water retention manifested by a large discrepancy between fluid intake and urinary output, and by a weight gain of 4.5 kilograms. Urinary osmolality was maximal (817 mOsm. per Kg.) on the first day of vasopressin administration, and thereafter slowly declined. Concomitant with the phase of water retention was a marked natriuresis, sufficient to produce a cumulative negative balance of sodium of 334

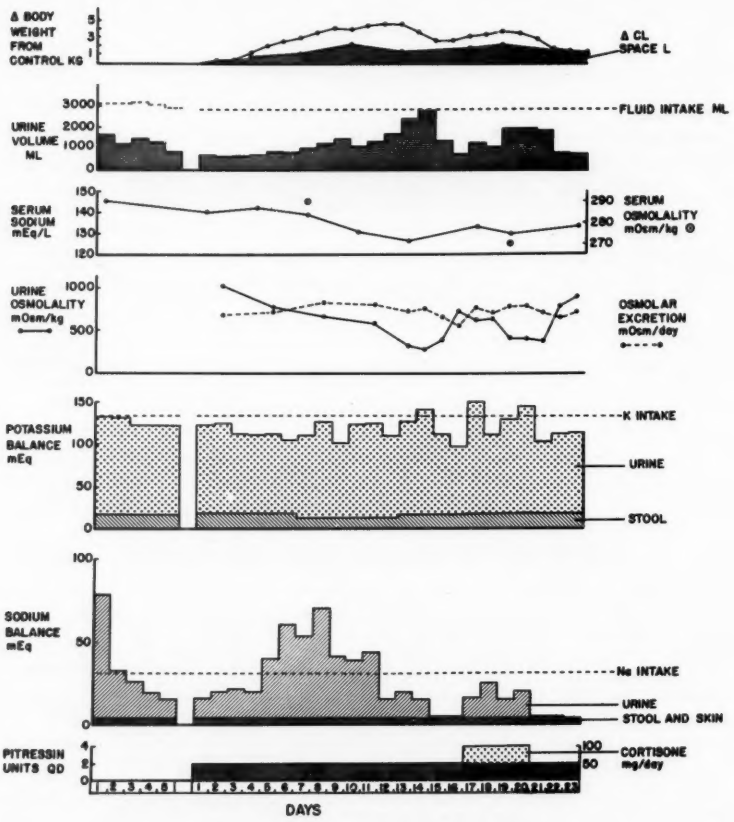

FIG. 2. Subject E.C. (Study #2). Effects of prolonged administration of vasopressin and a high water intake during a period of low-sodium diet.

milliequivalents during the first six days of the experimental period. A fall in serum osmolality and sodium concentration was observed during the period of water retention and sodium loss.

Following this initial phase, on days 7 through 10, water retention and natriuresis ceased and approximate balances of water and sodium were attained. Body weight was virtually constant during this period. Thereafter, on days 11 and 12, urine volume increased to more than 3 liters daily and there was a net loss of body water, manifested by a weight loss of 1.8 Kg. Urinary osmolality diminished to levels only slightly hypertonic to plasma, and sodium excretion diminished sufficiently so that a positive balance of sodium was achieved. The serum sodium concentration rose from 121 mEq. to 128 mEq. per liter during the period of diuresis.

A second period of water retention followed the phase of water loss (days 13 through 16, Fig. 1). In practically all respects this resembled the initial period of water retention. Despite an increase in vasopressin dosage to 4 units daily on day 15, and a 500-ml. increment in water intake initiated on day 20, water balance was subsequently attained at a body weight equivalent to that observed in the initial phase of water retention—approximately 5 Kg. above the control value. Urinary osmolality varied between 400 and 580 mOsm. per Kg. during the last nine days of the study—consistently lower than the levels observed on days 1 through 5 of the experimental period. It should be emphasized that although natriuresis did occur during the second phase of water retention in this study, there was a net positive balance of sodium during the last sixteen days of vasopressin administration.

The second study performed on Subject E.C. was identical to that just discussed, except that the sodium intake (32 mEq. per day) was relatively low. As shown in Figure 2, the responses to water and vasopressin administration were similar. Natriuresis was less marked in this study, as indicated by the observation that there was no net loss of body sodium over the 23-day period. Cortisone, 100 mg. per day, was given on days 17 through 20. Some increase in water excretion was evident during its administration, but the increase could not be clearly ascribed to an action of cortisone, since this diuresis was indistinguishable from that occurring spontaneously in study #1 or from that occurring on days 13 and 14 of study #2.

Vasopressin was given to another subject (L.P., with known hepatic cirrhosis) for a 16-day period. As shown in Figure 3, the results closely resemble those in Subject E.C. Cortisone was also given in this study, on days 11 through 14, but since water balance had been achieved prior to administration, its effect could not be clearly interpreted. A fairly marked hyponatremia was produced in this subject, as shown by a serum sodium concentration ranging between 114 mEq. and 117 mEq. per liter from day 4 throughout the remainder of the experimental period.

No consistent effect on potassium balance was observed during administration of vasopressin and water in either subject. Although urinary potassium excretion tended to be higher on days when the total urine volume was high, total potassium balances were not appreciably affected.

Changes in chloride space were determined in each of these studies. As shown in Figures 1–3, expansion of the chloride space accounted for a variable proportion of the total water retained. The discrepancy between total water balance and chloride space expansion indicates that a significant amount of the water retained was deposited within the intracellular fluid space.

Urinary osmolality was maximal on the first day of vasopressin adminis-

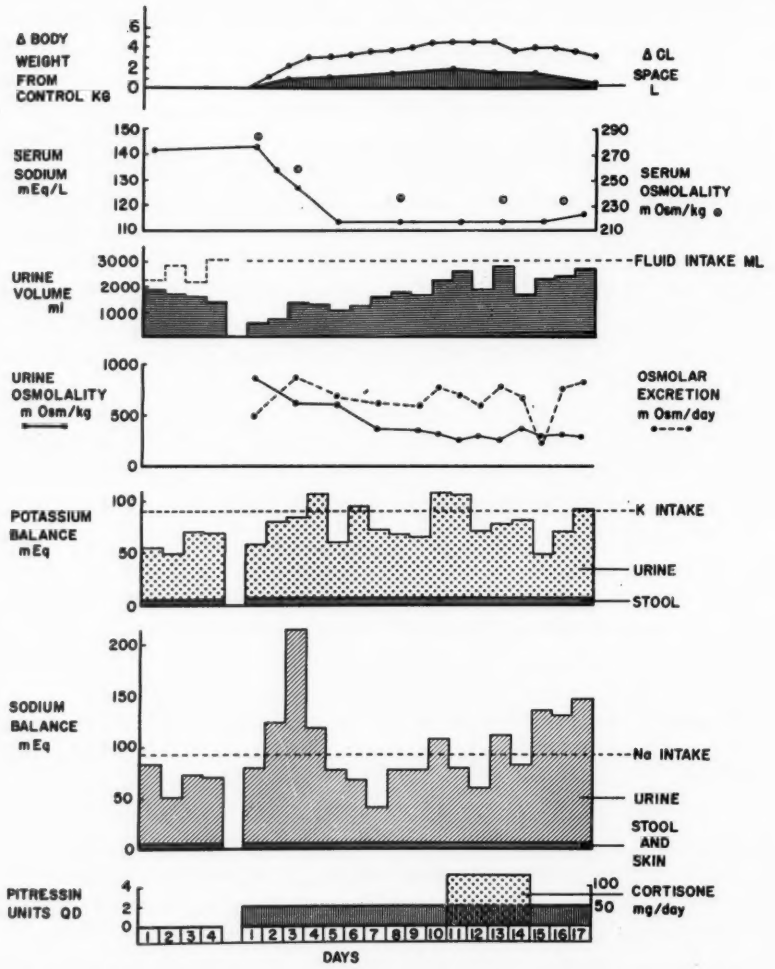

FIG. 3. Subject L.P. Effects of prolonged administration of vasopressin and a high water intake.

tration. The decline in urinary osmolality observed during the phase of water retention could be largely attributable to an increased rate of solute (sodium chloride) excretion. However, in each study the urinary excretion of sodium was minimal during the period of negative water balance. Consequently the marked decrease in urinary osmolality during this period was not the result of a high rate of solute excretion.

Each of these subjects experienced mild nausea and occasional headache during the period of maximal water retention. Subject L.P. manifested

moderate pitting edema of the ankles during this period. Edema was not observed in any other subject in this study. The presence of hepatic cirrhosis may account for this finding in Subject L.P.

Long-term administration of vasopressin to subjects receiving a normal or variable fluid intake

Subject H.W. received 4 units of vasopressin daily for a 29-day period (Fig. 4). A low-sodium intake (13 mEq. per day) was maintained throughout the study. The degrees of water retention and natriuresis were related to the level of fluid intake. These were slight at an intake of 1000 to 1200 ml. per day, whereas at an intake of 2000 ml. per day there was a striking weight gain and negative sodium balance (days 11 through 14). On two occasions (days 9 and 15) the infusion of mannitol resulted in a net loss of body water. In addition, several spontaneous diureses were observed during the course of the study (days 8, 18, 23 and 24). On several occasions the 24-hour urine was hypotonic to plasma, unlike the findings in Subjects E.C. and L.P., who during vasopressin administration always elaborated a urine which was at least iso-osmotic to plasma. As shown in Figure 4, cumulative sodium balance was positive through the last fourteen days of the experimental period. Since in this subject (H.W.) the caloric balance was negative throughout the study, water retention was underestimated by body weight changes. Consequently a plot of calculated body water balance has been included in Figure 4, and indicates a positive water balance throughout the period of vasopressin administration. In contrast to previous data (1), sodium restriction did not abolish the natriuretic response to overhydration in this subject, nor in Subject E.C. (study #2, Fig. 2) and one additional subject receiving a sodium intake of 20 mEq. per day. However in each study cited the degree of natriuresis was less pronounced than that observed in a subject receiving a high or a normal sodium intake.

A dose of 4 units of vasopressin was given daily to another subject (R.L.) whose fluid intake was unrestricted. Water retention was minimal and slight natriuresis was present only on days 2 through 5 of the experimental period. Serum sodium concentration decreased slightly, to a minimum of 131 mEq. per liter. On day 17 of vasopressin administration, at which time the calculated body water had increased approximately 1 liter above the control level, an oral water load of 1 liter in excess of his daily fluid intake was given. No immediate diuresis resulted, and a weight gain of 900 Gm. was recorded on that metabolic day. However on the following day, despite the continuance of vasopressin, urine volume rose to 2410 ml. and a weight loss of 1.74 Kg. occurred, indicating excretion of the acute water load as well as a portion of the previously retained body water.

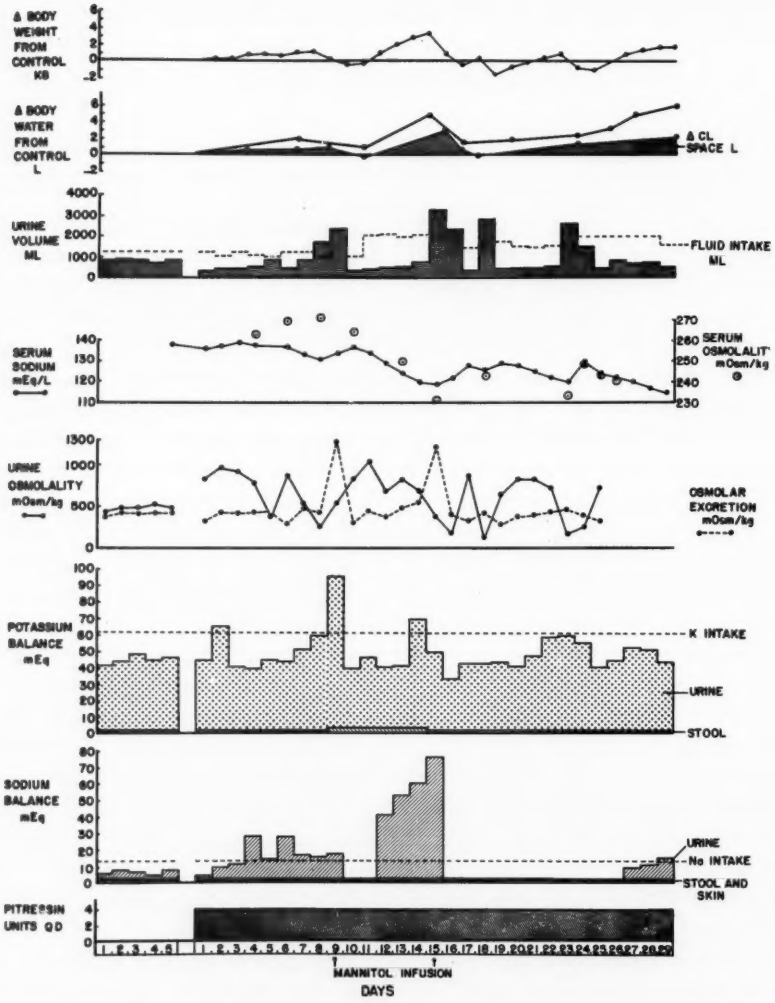

FIG. 4. Subject H.W. Effects of administration of vasopressin to a subject receiving a variable fluid intake and a low-sodium diet.

Short-term administration of vasopressin to subjects receiving a high fluid intake

Five studies were performed in subjects receiving 2 to 4 units of Pitressin daily, and a relatively high fluid intake (2000 to 3150 ml. per day). In each case symptoms of water intoxication (nausea, vomiting, headache and lethargy) necessitated discontinuance of the study after five to seven days.

The response to Pitressin was identical to that observed during the initial seven days in the studies on Subjects E.C. and L.P. (Figs. 1–3) and to that previously reported by Leaf and his associates (1). The detailed data from certain of these studies are included in a separate report from this laboratory (7).

DISCUSSION

The water retention and urinary sodium loss induced by administration of vasopressin and water appear to be self-limited in degree. In response to relatively prolonged administration of vasopressin a new steady state is achieved at a level of increased body water, slight to moderate sodium depletion, and persistent hyponatremia. This was a consistent response in all subjects receiving vasopressin for a period of more than ten days. A virtually identical response has been observed in dogs receiving a forced water intake and vasopressin for periods up to one month (8). In the latter study, water and sodium balances were re-established after an initial phase of water retention and sodium loss. As in human subjects, maximal urine osmolality was observed in the first several days following initiation of vasopressin, and thereafter declined.

Whereas a multiplicity of factors has been shown to influence renal concentrating ability, the present studies appear to be most comparable to previously published studies describing the effect of the state of bodily hydration on urinary concentration. It has been demonstrated that chronic ingestion of large volumes of water reduces the maximal urinary concentration achieved after the injection of vasopressin or after a period of water deprivation (9, 10). Similarly, chronic ingestion of water reduces the rate of abstraction of solute-free water from the renal tubules (T^cH_2O) achieved during solute diuresis (9, 10). The results of the present study suggest that the duration of the overhydrated state is a critical factor in determining the development of relative unresponsiveness to the injection of exogenous vasopressin. In several short-term studies, in which vasopressin was discontinued because of symptoms of water intoxication, overhydration of a degree greater than that produced in the prolonged studies failed to cause diuresis. Attainment of water balance was never observed prior to the seventh day after administration of vasopressin and water, and was not dependent upon the presence of marked overhydration. In the studies on Subjects H.W. and R.L. a reduced responsiveness to vasopressin, manifested by the production of hypotonic urine, was observed at a time when only slight water retention was present. It therefore appears that prolonged overhydration, to a moderate or slight degree, leads to a state in which renal concentrating ability, in response to the administration of vasopressin, is relatively impaired.

The mechanism whereby chronic overhydration influences renal concentrating ability remains unknown. In the present study the lack of correlation between solute excretion and urinary osmolality during the period of vasopressin administration indicates that the increased urinary volume evident at the time water balance was attained was not attributable to a solute diuresis. Similar conclusions were reached by Levinsky, Davidson and Berliner (8) in the previously cited studies on dogs. Although glomerular filtration rate was not measured in the present study, no correlation between filtration rate and urinary osmolality was observed in comparable studies on dogs (8). The glomerular filtration rate during the period of overhydration was, however, increased above the control levels (8). Since the influence of filtration rate on urinary concentration is probably mediated by the resulting effect on the rate of delivery of solute to the concentrating site (11), the lack of correlation between solute excretion and urinary osmolality in the present studies suggests that the fall in urinary osmolality observed during the course of prolonged vasopressin administration was not attributable to an augmented glomerular filtration rate.

According to our present understanding of the renal concentrating mechanism (12), at any given rate of solute excretion, urinary concentration is primarily dependent on the permeability of the distal and collecting-duct epithelium to water, and on the solute concentration within the renal medullary interstitium. The elaboration of a hypotonic urine in the presence of vasopressin (except perhaps in the case of extreme osmotic diuresis) suggests that the permeability of the tubular epithelium to water is impaired. In both the present study and that of Levinsky, Davidson and Berliner (8), hypotonic urine was observed in the presence of large doses of vasopressin, and in the absence of a significant solute diuresis. This suggests that chronic overhydration in some manner reduces the permeability of the renal tubular cells to water, although an effect on solute accumulation in the medullary interstitium can not be excluded. There is at present very little knowledge concerning those factors, other than vasopressin, which influence the permeability of the distal tubular system to water. It has been suggested that the adrenal steroids may exert such an action (13), but a direct effect of the adrenocortical hormones on water excretion has not been demonstrated, and the studies of Leaf and his co-workers have failed to demonstrate such a relationship in man (14). In the present study, no unequivocal effect of cortisone on urinary osmolality was apparent. The possibility that changes in the endogenous production of adrenal steroids may affect the renal response to prolonged vasopressin administration has in no sense been excluded by the present study, however.

The natriuresis which results from bodily overhydration has been previously described by others (1, 8, 15, 16). An increase in glomerular filtra-

tion rate (8), leading to both an increased filtered load of sodium and inhibition of aldosterone secretion (16), resulting in a reduced rate of tubular reabsorption of sodium, may contribute to the augmented rate of renal sodium excretion. The present study indicates that the natriuresis associated with water retention has certain well defined characteristics. In all instances sodium-losing was best correlated with an increasing body water content, rather than with its absolute level at any point in time. Thus, when water balance was attained, natriuresis ceased, despite the fact that overhydration amounting to greater than 5 per cent of the body weight had been produced. Diuresis, with a resultant net loss of body water, was associated with a low urinary excretion of sodium and with a positive balance of salt. Consequently sodium balance was always achieved when the subject attained a state of body water equilibrium. This observation seems pertinent to the clinical evaluation of hyponatremic states in which sustained endogenous vasopressin secretion is considered as the mechanism for the abnormal water balance (2–4). It suggests that significant bodily overhydration may exist without an associated sodium-losing tendency. It would be anticipated that renal sodium wasting would be evident only during periods of positive water balance, and consequently could be readily prevented by properly limiting fluid intake. The presence of continued sodium loss without progressive water retention would suggest either another etiology, or the presence of some complicating factor such as a disorder of renal or adrenocortical function. In the absence of complicating disease, therefore, overhydration secondary to sustained vasopressin action results in hyponatremia. This hyponatremia is secondary primarily to body fluid dilution and to a lesser extent to a loss of body sodium, but is not associated with a clinically significant degree of sodium chloride depletion.

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EFFECT OF TWO SYNTHETIC ESTROGENS ON THE LEVEL OF SERUM PROTEIN-BOUND IODINE IN MEN AND WOMEN WITH ATHEROSCLEROTIC HEART DISEASE

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ABSTRACT

Forty-three postmenopausal women with ischemic heart disease were treated with placebo or with 0.005–0.04 mg. of ethinyl estradiol daily for one to eleven months. Seventy-two men with ischemic heart disease were treated with placebo, or 0.01–0.50 mg. of ethinyl estradiol daily, or 10–80 mg. of Manvene daily for one to eleven months. The concentration of serum protein-bound iodine (PBI) was determined before and after initiation of treatment. The results were as follows: 1) The average increase in PBI concentration over the baseline level was 1.7 μg. per 100 ml. for women and 2.1 μg. per 100 ml. for men receiving ethinyl estradiol, and 2.0 μg. per 100 ml. for men receiving Manvene. 2) Ethinyl estradiol in dosages less than 0.005 mg. daily in women, or 0.02 mg. daily in men, was apparently no more effective than placebo in raising the PBI level. 3) Ethinyl estradiol in the dosages used was about 5 to 6 times more active in raising the PBI level in women than in men.

SINCE 1948 it has been known that the concentration of serum protein-bound iodine (PBI) in healthy women rises above normal values during pregnancy without manifestations of hyperthyroidism (1). The increased concentration becomes evident early in the first trimester, soon reaches a plateau, and falls to normal levels within one or two months after delivery. This seems to be an estrogenic effect, since the same PBI response can be obtained in both male and female patients by the administration of diethylstilbestrol, without causing hyperthyroidism (2–4). The minimal dose of exogenous estrogen required for such an effect has not been established.

We have been using estrogenic substances over long periods of time in the treatment of male and female patients with ischemic heart disease due to atherosclerosis. In a small sample of these patients, the serum PBI level was determined along with other laboratory data. Although the findings

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seemed significant, the study was not designed to answer the questions suggested by the laboratory results and further studies are indicated. Since we were using estrogenic preparations in relatively small dosages in men, and in extremely small dosages in women, we tried to determine: 1) whether it was possible to estimate the minimal dosage that could be expected to raise the PBI level, 2) whether there was a sex difference in the PBI response, and 3) whether the PBI response to ethinyl estradiol was different from that to Manvene[1] (3-methoxy-16α-methyl-1,3,5(10)-estratriene-16β,17β-diol).

MATERIAL AND METHODS

Forty-three postmenopausal women and 72 men with proven atherosclerotic heart disease were allocated at random to treatment in the out-patient clinic of the Los Angeles County Hospital. Of the 43 women, 16 were treated with placebo for one to eleven months, and 27 with ethinyl estradiol (0.005 to 0.04 mg. daily) for one to twelve months. Of the 72 men, 23 were treated with placebo, and 31 with ethinyl estradiol (0.01 to 0.50 mg. daily) for one to eleven months; 18 were treated with Manvene (10 to 80 mg. daily) for two weeks to ten months.

The serum PBI concentration was determined by the method of Barker *et al.* (5) modified by Ware (6). A baseline determination was always made before treatment with placebo or estrogens was started.

RESULTS

The data consist of one baseline PBI value and the first post-treatment PBI value in each case. Multiple post-treatment values were obtained in only a few cases—too small a number for statistical analysis.

Effect of treatment on PBI level in women (Table 1)

The 16 women who were allocated to placebo treatment had had a myocardial infarct from one to thirty-three months before the baseline PBI determination. The change in PBI within one to eleven months after the beginning of placebo treatment was not significant ($P > 0.3$). The PBI level dropped in 7 patients and rose in 9 patients during administration of placebo.

Twenty-six women who had had a myocardial infarct were given ethinyl estradiol orally in dosages ranging from 0.005 to 0.04 mg. daily; in 26 women the infarct had occurred from one to thirty-two months previously, and in 1 it had occurred forty-three months previously. There was no significant difference between the baseline PBI values in the placebo and the ethinyl estradiol groups ($P > 0.3$). The PBI level dropped in 4 women and rose in 23 during one to eleven months of treatment with ethinyl estradiol. The average rise from the baseline value was highly significant

[1] Manvene was supplied through the courtesy of Dr. I. C. Winter, G. D. Searle and Co.

TABLE 1. RESPONSE OF THE SERUM PBI LEVEL IN WOMEN TO TREATMENT WITH PLACEBO OR ETHINYL ESTRADIOL

Group	No. of patients	Time relation to treatment	PBI range (μg./100 ml.)	Avge. PBI level (μg./100 ml. ±σ)	p^{1}*	p^{2}	Avge. change from baseline PBI (μg./100 ml. ±σ)	p^{3}
Placebo	16	Before	3.8– 8.3	5.5±1.3				
		After	3.8– 8.8	5.9±1.3		>0.3	+0.5±1.2	
Ethinyl estradiol, 0.005–0.04 mg.	27	Before	3.0– 8.1	5.2±1.3	>0.3			
		After	3.4–11.9	6.9±1.2		<0.001	+1.7±2.0	<0.05

* p^{1} = comparison with PBI level before treatment with placebo.
p^{2} = comparison with PBI level before treatment with placebo or estradiol respectively.
p^{3} = comparison with change from baseline PBI level following treatment with placebo.

($P<0.001$). The difference between the average rises from the baseline value in the placebo and the ethinyl estradiol groups was significant ($P<0.05$).

Dose-response curves in women (Fig. 1)

The responses of the baseline PBI level to varying dosages of ethinyl estradiol in the 27 women were not significantly correlated ($P>0.1$). There

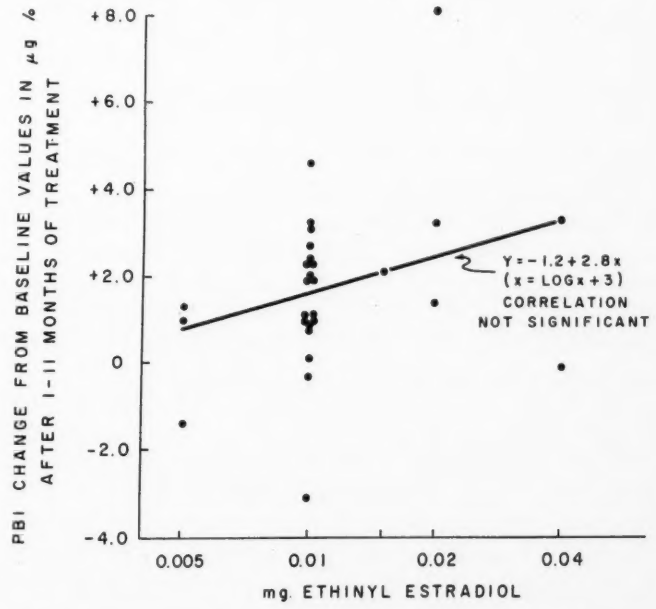

FIG. 1. Serum PBI response (μg. per 100 ml.) to varying dosages of ethinyl estradiol in 27 women with previous myocardial infarction. The dosage range of ethinyl estradiol was 0.005 to 0.04 mg. daily, administered for one to eleven months.

was a wide individual variation. Only 3 values were obtained at the 0.005-mg. dose level, none of which showed a significant difference from the PBI values in the placebo-treated group, whereas the average change for all doses was significantly different (Table 1).

Effect of treatment on PBI level in men (Table 2)

In 21 men who had had a myocardial infarct one to twenty-two months previously, and in 2 men who had had an infarct fifty-four and ninety-six months previously, the baseline PBI levels were determined before they were treated with placebo. There was no significant change between the baseline value and that observed during administration of placebo for one

TABLE 2. RESPONSE OF THE SERUM PBI LEVEL IN MEN TO TREATMENT WITH PLACEBO, ETHINYL ESTRADIOL OR MANVENE

Group	No. of patients	Time relation to treatment	PBI range (μg./100 ml.)	Avge. PBI level (μg./100 ml. ± σ)	p^1*	p^2	Avge. change from baseline PBI (μg./100 ml. ± σ)	p^3
Placebo	23	Before	3.8– 7.2	5.4±0.9				
		After	3.8– 7.0	5.5±0.8		>0.7	+0.1±1.5	
Ethinyl estradiol, 0.01–0.50 mg. daily	31	Before	3.1– 8.5	5.1±1.0	>0.2			
		After	3.5–11.9	7.3±1.9		<0.001	+2.1±2.0	<0.001
Manvene, 10–80 mg. daily	18	Before	3.3– 7.4	5.4±1.2	1.0			
		After	4.1–10.3	7.4±1.9		<0.001	2.0±1.0	<0.001

* p^1 = comparison with PBI level before treatment with placebo.
p^2 = comparison with PBI level before treatment with placebo, estradiol or Manvene respectively.
p^3 = comparison with change from baseline PBI level following treatment with placebo.

to eleven months ($P > 0.7$). The PBI level fell or remained unchanged in 10 men and rose in 13 men during placebo therapy.

Twenty-nine men who had had a myocardial infarct one to twenty-six months previously, and 2 men who had had an infarct thirty-eight and eighty-four months previously, were treated with ethinyl estradiol orally in dosages ranging from 0.01 to 0.50 mg. daily. The baseline values were not significantly different from those in the placebo group ($P > 0.2$). One to eleven months after the beginning of treatment with ethinyl estradiol there was a highly significant rise from the baseline PBI value ($P < 0.001$). The PBI level dropped in 3 men and rose in 28 men during ethinyl estradiol therapy. The difference between the PBI changes in the ethinyl estradiol placebo groups was highly significant ($P < 0.001$).

Seventeen men who had had a myocardial infarct from one to fifteen months previously, and 1 man who had had an infarct forty-five months previously, were treated with Manvene orally in a dosage of 10 mg. to 80 mg. daily. There was no difference in the baseline PBI values between the placebo and Manvene groups ($P = 1.0$). The rise from baseline PBI values

within one to ten months after the beginning of Manvene treatment was highly significant ($P < 0.001$). The PBI level rose during treatment in all the men. The difference between the PBI changes in the placebo and Manvene groups was highly significant ($P < 0.001$).

Dose-response curves in men (Fig. 2)

In the 31 men, the regression line for the dosage of ethinyl estradiol and the response of the PBI level was significant ($P < 0.01$) and conformed to the test of linearity. A log dose-response relationship was established for

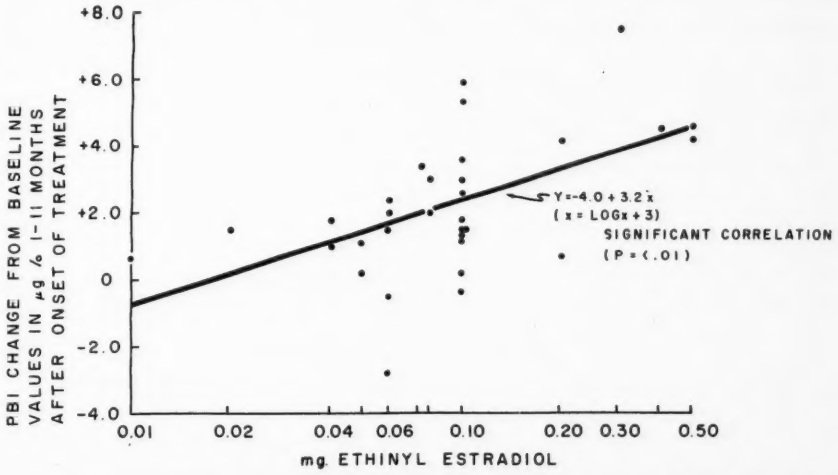

FIG. 2. Serum PBI response (μg. per 100 ml.) to varying dosages of ethinyl estradiol in 31 men with previous myocardial infarction. The dosage range of ethinyl estradiol was 0.01 to 0.50 mg. daily, administered for one to eleven months.

these men, within the range of dosage used. Each doubling of the dose resulted in a rise of PBI concentration by 0.95 μg. per 100 ml.

Comparison of dose-response curves for ethinyl estradiol in men and women (Fig. 3)

The regression lines for the PBI response to ethinyl estradiol for the 27 women (Fig. 1) and the 31 men (Fig. 2) were compared. It is realized that this comparison may not be warranted, since no significant dose-response relationship was demonstrated in women. However, linearity for the male regression seemed satisfactory, and for the female regression was at least not contraindicated, and both slopes were approximately parallel. When the results were described by a common regression line, their combined

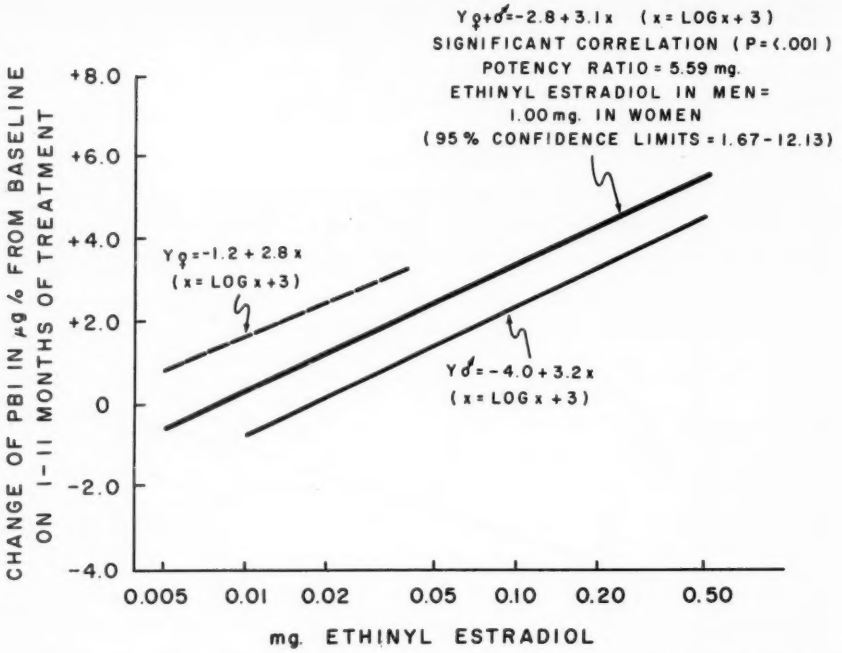

FIG. 3. Comparison of the effect of ethinyl estradiol on the serum PBI level in men and women.

values showed a highly significant correlation ($P < 0.001$). The potency ratio was therefore calculated.[2] From this computation, it was estimated that 5.59 times as much ethinyl estradiol was needed in men to cause a PBI response similar to that in women over the range of dosage used. This ratio cannot be expected to hold precisely, since the value is dependent upon clinical practice with regard to the size of the dose, and since there were wide 95 per cent confidence limits for our estimate (1.67 to 12.13).

Comparison of dose-response curves for ethinyl estradiol and Manvene in men

The regression lines showing the relationship between Manvene dosage and PBI response in 18 men conformed to the test of linearity, but there was no significant correlation ($P > 0.1$). For this reason a comparison with the regression for the PBI response to ethinyl estradiol in 31 men (Fig. 2) should be viewed with reservation. The calculated potency ratio at the

[2] $M = \bar{x}_1 - \bar{x}_2 - \dfrac{\bar{y}_1 - \bar{y}_2}{b}$.

average range of dosage used was 0.1 mg. of ethinyl estradiol to 29 mg. of Manvene, but this ratio was not precise and had extremely wide confidence limits.

DISCUSSION

Administration of estrogenic substances to human subjects results in an elevation of the serum PBI concentration. The effect of diethylstilbestrol has been well established (2–4); within the first three weeks after the beginning of treatment, the PBI level rises by an average of 1.8 μg. per 100 ml. above the control value and returns to the normal level in one to two months after cessation of therapy. The smallest dose of stilbestrol given was 20 mg. daily (3), and there was no further increase in PBI concentration with larger doses. The minimal effective dose of stilbestrol was not established. In a few patients, administration of 5 mg. of Premarin daily (3) has been observed to have a similar effect.

The findings of the present report indicate that the concentration of serum PBI can be raised by administration of ethinyl estradiol in men and women, and by Manvene in men.

The mechanism of the estrogen effect on the serum PBI level has been well discussed by others (4, 7). Apparently estrogen causes an increase in the thyroxine-carrying capacity of the binding proteins in serum. There may also be a shift in the ability of certain proteins to carry thyroxine, as demonstrated in pregnancy (8). Free thyroxine in the serum is thought to be unaffected or even diminished in pregnancy (9), thus accounting for the absence of clinical hyperthyroidism in pregnant or estrogen-treated patients with an elevated level of serum PBI.

In the present study, although a significant dose-response relationship could not be established for the women receiving 0.005–0.04 of ethinyl estradiol daily, the statistical regression suggested that the 0.005-mg. dosage had no more effect than placebo. However, dosages of 0.01 mg. and more caused a significant rise in the concentration of PBI. A significant log dose-response relationship was established for men receiving 0.01–0.5 mg. of ethinyl estradiol daily. The regression indicated that dosages less than 0.02 mg. daily probably could not be expected to have any more effect than placebo (although only 2 men were treated with doses below 0.02 mg.). Dosages above 0.02 mg. daily were effective in raising the average PBI level in the male group. Comparison of the PBI responses to ethinyl estradiol in men and women suggested that a dosage of 0.01 mg. daily in women could be expected to have the same effect as 0.05–0.06 mg. in men. Engstrom and Markardt (2) also reported that 25 mg. of diethylstilbestrol daily in women caused the same rise in the PBI level as did 100 mg. daily in men.

It is difficult to explain the greater biologic potency of estrogenic sub-

stances in women, and one can only speculate. Even though all the women in the present study were postmenopausal, perhaps they were still able to produce more endogenous estrogen from residual active ovarian tissue and the adrenals than the men could from the testes and the adrenals. Although not enough in itself to cause an elevation of PBI concentration, this endogenous estrogen may have had a "permissive action" on the carrying capacity of serum proteins, causing a measurable response with smaller doses of exogenous estrogen. Conversely, it may be that the increased amount of testosterone-like androgens in men, because of their known effect in decreasing the thyroxine-binding capacity of serum proteins (7, 10), partially inhibited or antagonized the action of estrogens. Hence a greater amount of exogenous estrogen was needed in men to have an effect similar to that in women. Another possibility is that there was a greater incidence of inadequately treated myxedema in the male group, resulting in interference with the PBI response (7). Although no clinically diagnosed cases of myxedema were included in this study, the lowest PBI concentration per 100 ml. of serum was 3.1 μg. in men and 3.0 μg. in women before treatment was instituted. In 8 (30 per cent) of the 27 women and 6 (19 per cent) of the 31 men the baseline values for PBI were below 4.6 μg. per 100 ml. before treatment with ethinyl estradiol. Therefore, the sex difference in estrogen effect cannot be explained on the basis of interference due to inadequately treated myxedema. On the other hand, these percentages may have a bearing on the occurrence of myocardial infarction in this series, since in only 11 per cent of a "sick control" group without ischemic heart disease were PBI values less than 4.6 μg per 100 ml. (11).

No significant dose-response relationship was established for men receiving 10–80 mg. of Manvene daily. However, the regression line seemed to indicate that even a 10-mg. dosage might be expected to raise the PBI level. The lowest effective dose was therefore not established.

In an additional 7 men treated with Premarin in an oral dosage of 0.625–5.0 mg. daily, the PBI level rose from the baseline value but the small size of the sample precluded statistical analysis.

In any clinical study involving relatively small dosages of estrogenic preparations in the treatment of coronary atherosclerosis, it is of great importance to determine whether such small quantities have a biologic effect. The doses of ethinyl estradiol most frequently used in this clinic are 0.10 mg. for men and 0.01 mg. for women. It has been demonstrated here that such small doses exert a significant effect on the serum PBI level, and that women are more responsive to estrogens than are men. It is unsafe to treat women with ethinyl estradiol in dosages much larger than 0.01 mg. daily because of the danger of induced uterine bleeding—a danger which is usually averted when the smaller dosages are used.

Acknowledgment

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DEPRESSION OF THE SERUM PROTEIN-BOUND IODINE LEVEL BY DIPHENYLHYDANTOIN*

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ABSTRACT

The mean serum protein-bound iodine (PBI) level in 36 cases of cerebral disease with seizures treated by administration of diphenylhydantoin was 3.81 $\pm$0.14 (S. E.) μg. per 100 ml. This was significantly lower than the corresponding mean PBI level in 12 cases of cerebral seizure not so treated, *viz.*, 5.39 $\pm$0.16 μg. per 100 ml. Despite the occurrence of PBI levels as low as 2.4 μg. per 100 ml. in the diphenylhydantoin group, there was no clinical evidence of hypothyroidism in any of the patients and there were no significant alterations in the other parameters of thyroid function. In 7 of 8 euthyroid subjects, diphenylhydantoin also depressed the serum PBI concentration. The mechanism of action of diphenylhydantoin is apparently extrathyroidal, since this drug depressed the PBI level in hypothyroid patients maintained with a constant dosage of desiccated thyroid. Diphenylhydantoin added *in vitro* consistently increased the uptake of I^{131}-labeled 3,5,3′-triiodothyronine by red blood cells incubated with plasma. This effect was progressively diminished by the addition of increasing quantities of stable *l*-thyroxine to the incubating mixture. These data are compatible with the thesis that diphenylhydantoin depresses the level of serum PBI by interfering with the binding of thyroxine by plasma proteins.

IN THE course of investigating neuro-endocrine relationships in patients with cerebral disease, it soon became apparent that the serum protein-bound iodine (PBI) level was depressed in a large number of patients with seizures who were being treated with the anticonvulsant, diphenylhydantoin (Dilantin Sodium®). The following studies were pursued with the hope

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of elucidating the mechanism whereby this depression of the PBI level is effected.

METHODS

Determinations of serum PBI concentration were performed at the Bio-Science Laboratories by the method of Barker *et al.* (1). Serum cholesterol levels (2), thyroidal 24-hour I^{131} uptakes (3), and basal metabolic rates (4) were determined by standard methods. The erythrocyte uptake of I^{131}-labeled triiodothyronine (T_3), a procedure introduced by Hamolsky *et al.* (5), was performed according to the modification of Friis (6). Red blood cells and plasma were combined to a standard hematocrit of 50 per cent; no correction to 100 per cent hematocrit was made. Thyroidal clearance of I^{131} was estimated on the basis of direct determination of the net gain in thyroidal radioactivity over a 45-minute period starting one hour after the oral administration of 8 to 30 μc. of I^{131}. Thyroidal uptakes were estimated by the difference between the total activity counted by the collimated Geiger-Mueller tube and that counted after shielding the thyroid area with a lead brick 2 cm. thick. The mean plasma I^{131} concentration during this period was computed on the basis of serial plasma determinations obtained at 15-minute intervals.

RESULTS AND INTERPRETATIONS

Effect of diphenylhydantoin on the serum PBI level in cases of cerebral disease with seizures (Fig. 1)

It was necessary to determine whether the depression of PBI concentra-

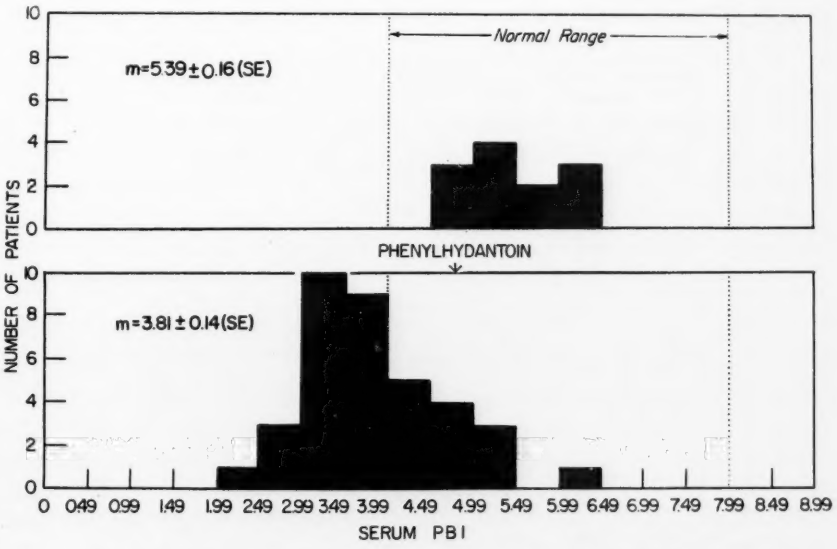

FIG. 1. Frequency-distribution plots of serum PBI levels in cases of cerbral disease with seizures. The *lower* graph shows the values in 36 patients treated with diphenylhydantoin. The *upper* graph shows the values in 12 patients not so treated.

tion observed in preliminary observations was due to the primary central nervous system disease or to the anticonvulsant drugs employed. Therefore, patients seen in the Seizure Clinic were divided into two groups: *a*) those being treated with diphenylhydantoin alone or in combination with other classes of drugs, and *b*) those *not* under treatment with diphenylhydantoin (*i.e.*, being treated with other anticonvulsants or receiving no medication). All patients in the diphenylhydantoin group received the drug in the usual therapeutic dosage (0.3 to 0.6 Gm. per day).

The mean PBI value in 36 seizure patients receiving diphenylhydantoin was 3.81 ± 0.14 (S. E.) μg. per 100 ml., whereas the mean PBI value in 12 seizure patients not receiving diphenylhydantoin was 5.39 ± 0.16 μg. per 100 ml. The difference between the means was statistically significant at the 1 per cent level (Fig. 1). The evidence would thus suggest that the observed depression of PBI values was a function of the treatment rather than of the underlying disease.

Effect on chemical determination of PBI. The possibility was investigated that diphenylhydantoin, like mercury (7), might somehow interfere with the chemical determination of PBI. However, the *in vitro* addition of diphenylhydantoin to serum, in amounts comparable to circulating levels during therapy (8) did not cause any significant change in PBI values (Table 1). Furthermore, when plasma from a patient treated with diphenylhydantoin was mixed in equal proportion with the plasma of an untreated

TABLE 1. EFFECT ON THE DETERMINATION OF SERUM PBI CONCENTRATION OF *in vitro* ADDITION OF (I) DIPHENYLHYDANTOIN OR (II) SERA OF PATIENTS TREATED WITH DIPHENYLHYDANTOIN

Patients A and C: euthyroid, not taking diphenylhydantoin.
Patient B: hyperthyroid, not taking diphenylhydantoin.
Patients D and E: euthyroid, taking diphenylhydantoin.

	PBI (μg./100 ml.)
I. *Addition of diphenylhydantoin*	
A (1 ml.)	4.2
A (1 ml.) +30 μg. diphenylhydantoin	4.2
B (1 ml.)	11.1
B (1 ml.) +30 μg. diphenylhydantoin	11.7
II. *Addition of serum from diphenylhydantoin-treated patients*	
C (1 ml.)	4.6
D (1 ml.)	3.0
C (0.5 ml.) +D (0.5 ml.) (anticipated)	3.8
C (0.5 ml.) +D (0.5 ml.) (observed)	3.7
B (1 ml.)	11.1
E (1 ml.)	3.1
B (0.5 ml.) +E (0.5 ml.) (anticipated)	7.1
B (0.5 ml.) +E (0.5 ml.) (observed)	7.5

patient, the PBI concentration in the mixture represented the simple arithmetic mean of the component PBI values. This observation makes it unlikely that a circulating metabolite of dipheylhydantoin interfered with the chemical determination.

Other thyroid function tests in cases of cerebral disease with seizures, treated with diphenylhydantoin (Table 2)

Even among the diphenylhydantoin-treated patients with very low PBI levels there was no clinical evidence of myxedema. The only symptoms compatible with hypothyroidism were listlessness and drowsiness, and these could be attributed to the sedative properties of diphenylhydantoin as well as to the barbiturates which many of these patients were taking concomitantly. None of these patients had clinically apparent goiters.

TABLE 2. THYROID FUNCTION TESTS IN CASES OF CEREBRAL DISEASE WITH SEIZURES, TREATED WITH DIPHENYLHYDANTOIN

	Serum PBI (μg/100 ml.)	Thyroidal 24-hr. I^{131} uptake (% of dose)	Thyroidal clearance of I^{131} (ml./min.)	Serum cholesterol (mg./100 ml.)	BMR (%)
Mean	3.81	38.5	22.0	222	− 8.2
±S.E.	0.14	1.1	9.5	18	3.4
No. of determinations	36	14	6	14	10

In Table 2 are summarized the available data on other parameters of thyroid function in this group. The mean thyroidal 24-hour I^{131} uptake, the mean thyroidal plasma clearance of I^{131}, and the mean basal metabolic rate were all within normal limits. PBI^{131} determinations performed in several patients twenty-four hours after the administration of I^{131} dose were well within normal limits.

Sequential studies of thyroid function in euthyroid and treated hypothyroid subjects during administration of diphenylhydantoin

Euthyroid subjects (Table 3, Fig. 2). Serial determinations of serum PBI levels were performed in 3 normal subjects and 5 neurologic subjects, before and during the administration of diphenylhydantoin (0.3–0.4 Gm. per day). With 1 exception (W.S.), they showed a fall in PBI concentration after starting diphenylhydantoin therapy. In the 3 normal subjects, there was a prompt return of the PBI level to control values after discontinuation of diphenylhydantoin. In Subject T.G., after an initial fall, the concentration of PBI returned to pretreatment levels despite continued

TABLE 3. SEQUENTIAL STUDIES OF THYROID FUNCTION BEFORE AND DURING ADMINISTRATION OF DIPHENYLHYDANTOIN IN EUTHYROID SUBJECTS

Subject (age, sex, diagnosis)	Duration of d.p.hydantoin admin.	PBI* (μg./100 ml.)	Thyroidal 24-hr. I^{131} uptake (% dose)	Serum cholesterol (mg./100 ml.)	Thyroidal I^{131} clearance (ml./min.)	BMR (%)
L.F. 30, M, normal	0 2 wks.	5.7 4.4	— —	— —	— —	— —
J.O. 32, M, normal	0 2 wks.	4.8 4.0	— —	— —	— —	— —
M.N. 26, M, normal	0 2 wks.	5.5 4.3	31 39	244 233	— —	−19 —
E.S. 13, M, epilepsy	0 2 wks.	6.1 4.4	23 28	268 252	— —	−22 −16
J.M. 67, M, trigem. neuralgia	0 2 wks.	4.1 3.7	18 17	268 284	12 19	−23 −14
C.R. 31, F, epilepsy	0 3 wks.	7.4 5.3	— 34	— 233	— 10	— −10
T.G. 48, F, epilepsy	0 1 wk. 11 wks.	5.2 4.2 5.3	— — —	— — —	— — —	— — —
W.S. 62, M, cardiovasc. accident	0 2 wks.	5.4 5.2	30.9 33.0	220 247	14 8	−13 −13

* Serial PBI values represented in greater detail in Figure 3.

administration of diphenylhydantoin. Studies of other parameters of thyroid function are shown in Table 3. On the basis of these data, there were no significant changes in the results of other thyroid function tests as a result of short-term administration of diphenylhydantoin.

Hypothyroid patients maintained with desiccated thyroid (Fig. 3). Five patients with documented hypothyroidism, maintained with a constant replacement dosage of desiccated thyroid, were given diphenylhydantoin (0.3–0.4 Gm. daily) for two weeks (Table 4). All exhibited a prompt fall of the serum PBI level (Fig. 3) after the institution of diphenylhydantoin therapy, and an equally prompt rise toward pretreatment values after discontinuation of the drug. Because of the improbability of significant residual thyroid function in these patients, it would appear that the diphenylhydantoin effect is exerted primarily at some extrathyroidal site.

Effect of diphenylhydantoin on the erythrocyte uptake of I^{131}-labeled triiodothyronine (T_3)

The possibility was investigated that diphenylhydantoin depressed the serum PBI level by competing with thyroxine for specific binding sites on

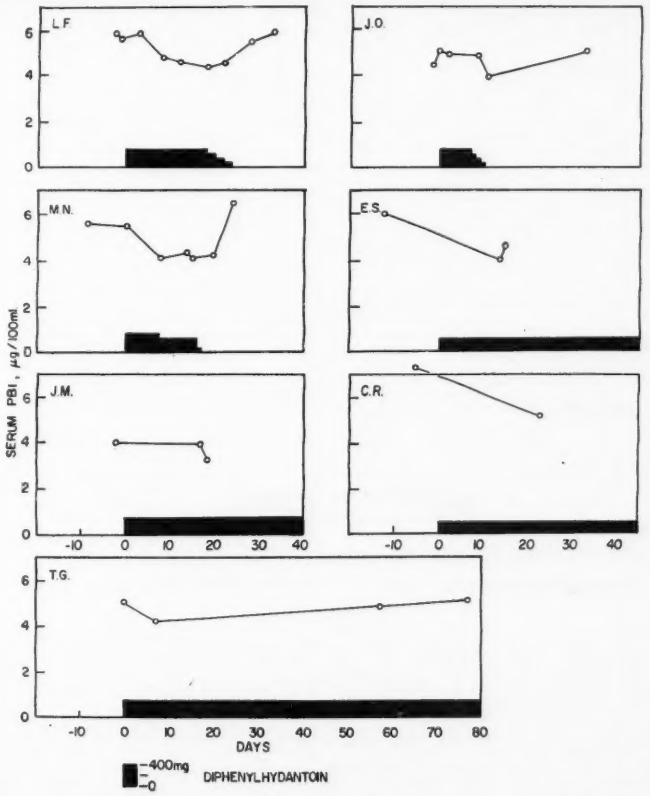

FIG. 2. Effect of diphenylhydantoin on serum PBI level in 7 euthyroid subjects. In an eighth euthyroid subject (W.S., Table 3), there was no significant fall in the PBI level.

plasma proteins. A similar mechanism has been postulated for aspirin and dinitrophenol(9). The uptake of I^{131}-labeled T_3 by human red blood cells in an incubation mixture of plasma and red cells appears to be largely a func-

TABLE 4. HYPOTHYROID PATIENTS MAINTAINED WITH CONSTANT DOSAGE OF DESICCATED THYROID

Patient	Age & sex	Diagnosis	Maintenance dosage of desicc. thyroid (mg./day)
L.G.	38 M	Post-thyroidectomy for carcinoma of thyroid	180
C.L.	44 F	"Burnt-out" acromegaly	180
L.L.	36 F	Cretin	60
M.D.	48 F	Idiopathic hypothyroidism	150
D.J.	36 F	Post-thyroiditis	60

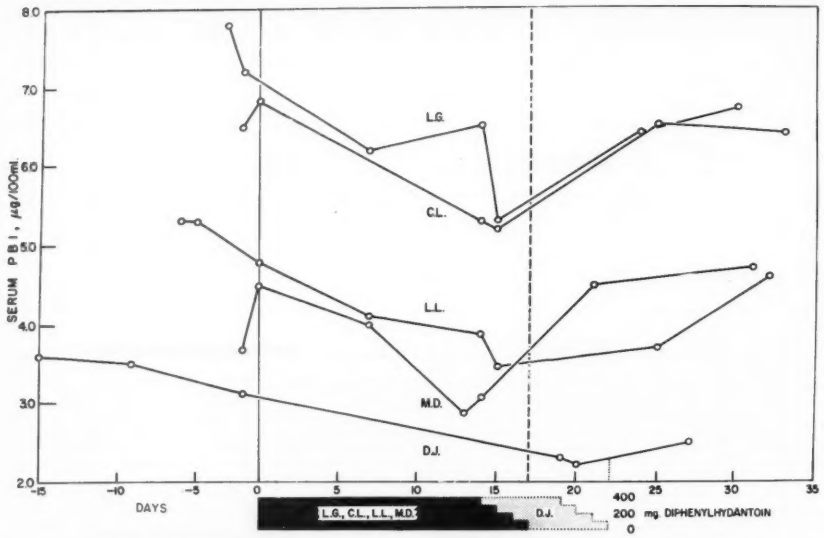

FIG. 3. Effect of diphenylhydantoin on serum PBI level of 5 hypothyroid patients maintained with the same dose of desiccated thyroid throughout the study.

tion of the available plasma thyroxine-binding sites(10). The erythrocyte uptake of radioactive T_3 from incubation mixtures containing graded amounts of diphenylhydantoin was thus determined. Figure 4 illustrates the striking rise in uptake observed after the addition of as little as 12.5 μg. of diphenylhydantoin per ml. of incubation mixture (5×10^{-5} M/L). This concentration is within the observed range of diphenylhydantoin plasma concentration in patients treated with this drug (8).

In order to rule out the possibility of a direct effect on erythrocytes rather than plasma, the following experiment was performed. Carefully washed red cells were incubated with physiologic salt solution. The addition of diphenylhydantoin (100 μg. per ml. of incubating mixture) failed significantly to increase the uptake of labeled T_3 by the red cells (control, 54.7 per cent; diphenylhydantoin, 54.9 per cent).

The possibility remained that diphenylhydantoin blocked the attachment of labeled T_3 to some locus other than plasma protein thyroxine-binding sites. If this were the case, one would anticipate that in the presence of excess thyroxine and consequent saturation of the binding sites, diphenylhydantoin would still be capable of increasing the red cell uptake of radioactive triiodothyronine. On the other hand, if diphenylhydantoin effectively competed with triiodothyronine at thyroxine-binding sites, then an excess of thyroxine should be able to block the diphenylhydantoin

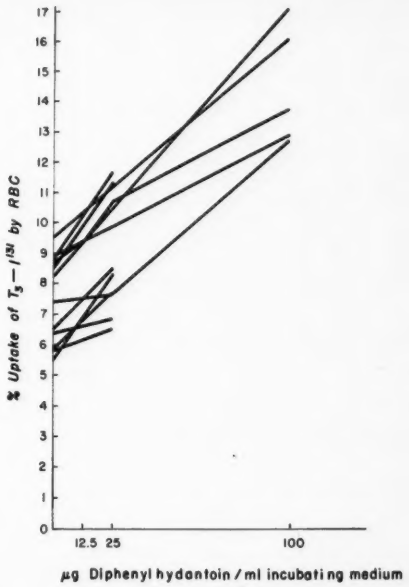

FIG. 4. Effect of diphenylhydantoin on the thyroidal uptake of I^{131}-labeled triiodothyronine by red blood cells incubated in plasma.

effect. Accordingly, progressive increments of stable *l*-thyroxine were added both to flasks containing 87 μg. of diphenylhydantoin per ml. of incubating mixture and to control flasks containing the red-cell uptake system without added diphenylhydantoin (Fig. 5). It can be seen from the resulting curves that elevated levels of thyroxine did in fact diminish the diphenylhydantoin effect. The data are thus consistent with the concept that diphenylhydantoin interferes with the binding of T_3 at the thyroxine-binding sites of plasma proteins.

DISCUSSION

The *in vivo* studies indicate that diphenylhydantoin depresses the serum PBI level by some extrathyroidal mechanism. Serial determinations in euthyroid subjects demonstrated a depression of the PBI level in most, but not all, when they were treated with diphenylhydantoin. The lack of a sustained fall in PBI concentration in some subjects may have been due either to a masking of the effect by fluctuations in the PBI level (11), or to a dosage inadequate for those particular individuals. In view of the results of the cross-sectional studies on patients with cerebral-disease seizures and the sequential studies in euthyroid and thyroid-treated hypothyroid subjects, however, there is a clear indication of the depressing effect of diphenylhydantoin on the level of serum PBI. On the basis of the available evidence, there did not appear to be any significant change in the other

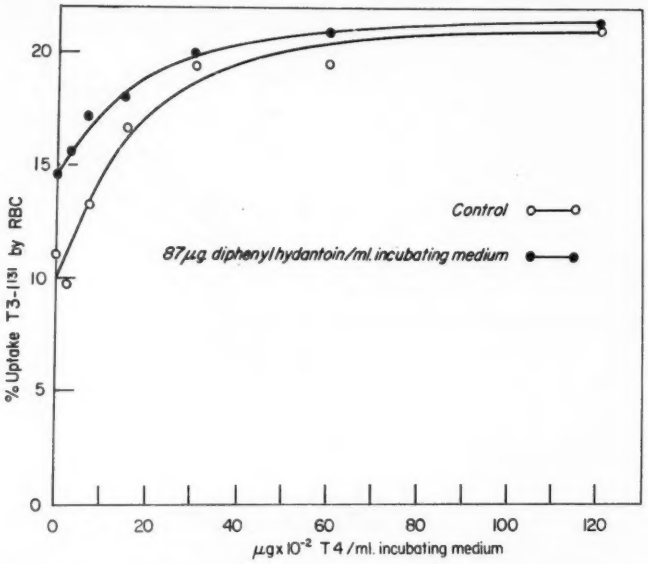

FIG. 5. Influence of *l*-thyroxine on the diphenylhydantoin effect on erythrocyte uptake of I^{131}-labeled *l*-triiodothyronine.

parameters of thyroid function, and the patients appeared to be clinically euthyroid.

The *in vitro* data derived from erythrocyte uptake studies provide further evidence for the extrathyroidal effect of diphenylhydantoin and suggest that this drug interferes with the binding of thyroxine by plasma proteins. Christensen (10) has postulated a similar mode of action for aspirin and dinitrophenol to account for the ability of these substances to depress the PBI level. Unlike dinitrophenol and aspirin, however, diphenylhydantoin does not decrease the thyroidal uptake of I^{131} nor increase the metabolic rate (12, 13).

Chromatographic studies[1] in a patient receiving diphenylhydantoin indicated a normal distribution of radio-iodinated amino acids in the plasma. This observation, as well as the absence of goiter in these patients, indicates that there was no intrathyroidal block in the synthesis of thyroid hormone. Furthermore, the level of PBI^{131} and the thyroidal clearance of I^{131} were normal. It would therefore seem safe to conclude that the quantity of hormonal iodine reaching the tissues was normal in a situation of steady-state. If one disregards the possibility that diphenylhydantoin may change the distribution space of PBI, the assumption would be that the turnover rate of PBI is increased.

[1] Kindly performed by Dr. Sidney Werner.

Since hydantoin, a substance which lacks the diphenyl substituent, had no effect on the erythrocyte uptake test, it would appear that the diphenyl moiety is important in producing the *in vitro* effects of diphenylhydantoin. It may be of interest that aspirin and dinitrophenol are also aromatic structures.

The diphenylhydantoin effect may be analogous to the situation in some patients with a nephrotic syndrome in whom there is a depression of the level of thyroxine-binding globulin. On the basis of the kinetic considerations, Robbins and Rall (14) postulated that the level of unbound thyroxine in such patients is normal. They also believed that the euthyroid status of these patients could be attributed to the normal levels of unbound thyroxine. Because of the reduced levels of thyroxine-binding globulin, the red blood cell uptake of I^{131}-labeled triiodothyronine is elevated in the nephrotic syndrome (15). Diphenylhydantoin may also serve to diminish the binding sites available to thyroxine by competing with thyroxine for the same binding site.

Acknowledgments

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URINARY 6β-HYDROXYCORTISOL IN ADRENOCORTICAL HYPERFUNCTION*†

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ABSTRACT

Three patients with abnormal amounts of 6β-hydroxycortisol in the urine are presented. In 2 of these patients, adrenal hyperplasia was found at autopsy. The third patient had Cushing-like clinical symptoms, and an adrenal adenoma was found at operation; in this case, although the excretion of 6β-hydroxycortisol was elevated, the urinary alpha-ketolic steroid pattern was otherwise normal. Technics for the isolation of 6β-hydroxycortisol are described.

THE present study shows that 6β-hydroxycortisol (4-pregnene-6β, 11β,17α,21-tetrol-3,20-dione) was one of the major urinary alpha-ketolic steroid excretory products in 3 patients with pathologic conditions of the adrenal cortex. Burstein and co-workers originally isolated this substance from human urine in 1954 (1). Of our 3 patients, 2 with adrenocortical hyperplasia also had elevated excretion of 17-hydroxycorticoids and 17-ketosteroids as determined by routine procedures. The third patient had an adrenal adenoma, subsequently proved by surgery; urinary 17-ketosteroid and 17-hydroxycorticoid outputs were normal but the output of 6β-hydroxycortisol was elevated. Intravenous administration of corticotropin (ACTH) in this case caused an increase in the excretion of 6β-hydroxycortisol from 0.6 to 1.2 mg. per twenty-four hours.

Methods for isolation, a description of the properties of 6β-hydroxycortisol, and a comparison of the amounts found in these 3 patients and in normal subjects are presented in this paper. The amounts of the major polar urinary alpha-ketolic steroids separated by paper chromatography are also presented. Incomplete extraction from aqueous solutions (including urine) and poor chromatographic separation with procedures heretofore

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utilized have resulted in 6β-hydroxycortisol being overlooked because of its great polarity.

CASE HISTORIES

Case 1

W.K. was a 68-year-old male with extensive metastases from carcinoma of the prostate, and severe hypokaliemic alkalosis. The prostatic carcinoma appeared to have developed first. The urinary excretion of aldosterone and of glucocorticoids as measured by paper chromatography was greatly increased, and at autopsy bilateral adrenal hyperplasia was found. Despite the elevated glucocorticoid excretion, the only clinical findings suggesting Cushing's syndrome were hyperglycemia and glycosuria. Limited metabolic studies revealed renal wasting of potassium, the constant excretion of an acid urine, and continued inability to concentrate the urine. A detailed description of the clinical and pathologic considerations in this subject are provided in a previous report (2).

Case 2

L.W. was a 36-year-old woman with an unusual pattern of Cushing's syndrome associated with a functioning islet-cell carcinoma of the pancreas. She had the moon facies hypertension and striae of Cushing's syndrome, with a right upper abdominal mass and an elevated 17-ketosteroid excretion of 28.1 mg. per twenty-four hours. At operation a large mass was found, which originated from the region of the right adrenal gland. Local and hepatic metastases were present. Biopsy of the mass showed a poorly differentiated carcinoma. Postoperatively, hypoglycemia developed and progressed in severity to the point of constant convulsions and death despite large amounts of glucose given orally and intravenously. During this postoperative period, the urinary excretion of adrenal steroids increased markedly and the blood insulin level was five to ten times the normal value. Marked adrenal hyperplasia was found at necropsy, associated with a malignant carcinoma of the pancreas. A detailed description of the clinical and pathologic aspects of this case are provided in a previous report (3).

Case 3

M.G., a 38-year-old Caucasian woman, was admitted to the Hospital of the University of Pennsylvania on September 23, 1959 by Dr. Edward Rose for evaluation. Studies were initiated at a nearby hospital four weeks previously because of delayed wound healing. Six weeks previously she had fallen from a chair and sustained a laceration of the right pretibial region. Attempts to suture the wound were unsuccessful and her physician at that time remarked about the extreme friability of the skin. Over a period of six years prior to this admission she had noticed thinning and reddening of the skin, thinning of her hair accompanied by generalized mild hirsutism, and the development of a fat pad between her scapulae. Delayed wound healing and easy bruisability had been encountered previously in association with lacerations of the lower leg which required four months on one occasion and twelve months on another for healing. From the time of her menarche at the age of 12 years, her menses had been regular until, concurrently with the development of other symptoms, she noted oligomenorrhea and shortening of the menstrual cycle and finally complete amenorrhea. She had had only one menstrual period in the two years prior to admission. She reported a tendency to moodiness and depression but no change in libido. Her body weight decreased from 130 to 111 pounds. She had noticed that her fingernails and toenails had been extremely fragile for a period of seven years. Physical findings were: blood pressure varying from 120/80 to 170/110 mm. Hg in

the right arm in a recumbent position; heavy jowls, but not the typical moon facies; and thin, red, shiny skin with increased pigmentation over the lower legs but no definite striae. There was a large interscapular hump. The thyroid and breasts were normal in size and consistency. The pelvic viscera were normal and the pubic escutcheon was of the female type, There were multiple thin atrophic scars over the lower extremities, and an ulcer (6 cm. in diameter) over the right pretibial area. The feet were warm and dry. The findings on neurologic examination were within normal limits. Results of clinical laboratory studies were: hemoglobin level 15.9 Gm. per cent; leukocyte count 15,500 per cu.mm. with 64 per cent neutrophils, 4 per cent monocytes and 32 per cent lymphocytes; level of blood urea nitrogen 23 mg. per 100 ml.; fasting blood sugar 77 mg. per 100 ml. with a slightly high glucose tolerance curve; serum calcium 9.8 mg. per 100 ml.; phosphorus 3.2 mg. per 100 ml.; alkaline phosphatase activity 5 (Jones-Rhinehart) units; and tubular phosphate reabsorption 83 per cent. The concentration of serum sodium was 140 mEq., potassium 4.7 mEq. and CO_2 23.7 mEq. per liter. Bleeding time, clot retraction, coagulation time, platelet count, eosinophil count, hematocrit, BMR, urinalysis findings and a lupus erythematosus cell preparation were normal. Serum protein concentration was 6.15 Gm. per cent with a serum albumin level of 4.10 Gm. per cent despite evidence of poor protein nutrition. The protein electrophoretic pattern was normal.

The 24-hour urinary excretion of 17-hydroxycorticoids was 2.8 mg., and that of 17-ketosteroids was 5.6 mg. and 4.4 mg. on two occasions. Following intramuscular administration of 50 units of corticotropin (Acthar gel) there was a normal response; the urinary 17-hydroxycorticoids rose to 11.2 mg. and the 17-ketosteroids to 9.7 mg. per twenty-four hours. Studies of the urinary alpha-ketolic steroids by special chromatographic technics (as discussed in the text) showed abnormal amounts of 6β-hydrocortisol in the preoperative urine collections.

All roentgenograms showed generalized demineralization, several changes in the bone being suggestive of pseudofracture. The sella turcica was normal in size and had not changed since the films taken in January 1957. X-ray examination of the teeth showed a number of cavities. The lamina dura was present. Perirenal carbon-dioxide insufflation (4) did not reveal an adrenal tumor on either side. Intravenous urograms showed a distortion of the left upper middle calyceal system.

On October 9, 1959, a transabdominal exploratory operation revealed a tumor (1.5×2 cm.) in the left adrenal gland. The right adrenal was normal. All tissues were extremely friable. A left adrenalectomy was carried out and, because of a small tear in the splenic capsule, an elective splenectomy was performed.

Following operation the patient received prophylactic hydrocortisone which was gradually reduced in dosage and then discontinued without evidence of adrenal insufficiency prior to her discharge from the hospital on October 31, 1959. The operative wound healed normally. The leg ulcer also improved, and by the time of discharge it measured 3.5 cm. in diameter. Three weeks following discharge the patient was readmitted because of a subdiaphragmatic collection of fluid, which was drained. Pancreatitis appeared to be a complicating factor. The response to standard surgical procedures was slow, and supportive measures were initiated, with emphasis on nutritional supplementation. She was discharged in late February 1960 with a healing sinus of the left loin. During the November-February admission the 24-hour excretion of urinary 17-ketosteroids was 2.7 mg., and 17-hydroxycorticoids 2.1 mg. To date there has been a definite decrease in the size of the nuchal fat pad, and improvement in her facial appearance with a decrease in the buccal fat pads. The leg wound has healed, her hair is of normal texture, her nails are of normal structure, and she had her first menstrual period

in March 1960. Her disposition has improved considerably and her husband has noted a return to her pre-illness personality.

The tumor, on gross examination, was 3 cm. at its greatest diameter. The adrenal gland, with a very thin cortex, covered the tumor and extended beyond its edges (Fig. 1). The cut section of the tumor was bright yellow with dark brown areas, but both colors became darker on exposure to air. The microscopic sections showed a well encapsulated tumor which consisted for the most part of polygonal cells with clear vesicular

FIG. 1. Bisected functioning adenoma removed by left adrenalectomy from a 38-year-old woman (M.G.). A thin layer of adrenal cortex covered the tumor. The cut surface was bright yellow with brown areas, but both colors became darker when exposed to air.

cytoplasm and small rounded vesicular nuclei. The cells were arranged in small clusters bound by collagen bands. In some areas the cells had finely granular eosinophilic cytolasm (Fig. 2).

METHODS

The pH of the urine was adjusted to 4.5 with acetic acid plus sodium acetate, and the urine was then incubated for forty-eight hours with β-glucuronidase (Ketodase, 300 units per ml.). Double extraction was carried out with half volumes of ethyl acetate and chloroform. The organic phases were washed successively twice with tenth volumes of saturated sodium bicarbonate and water, and then evaporated in a vacuum. Nadel and associates (5) used ethyl acetate in their work. Chloroform was included here to extract the less polar metabolites. Subsequent work has shown that the addition of sodium sulfate to the urine before extraction results in the removal of an additional increment of substances measured by the Porter-Silber phenylhydrazine reaction. This has been reported by Katz *et al.* (6).

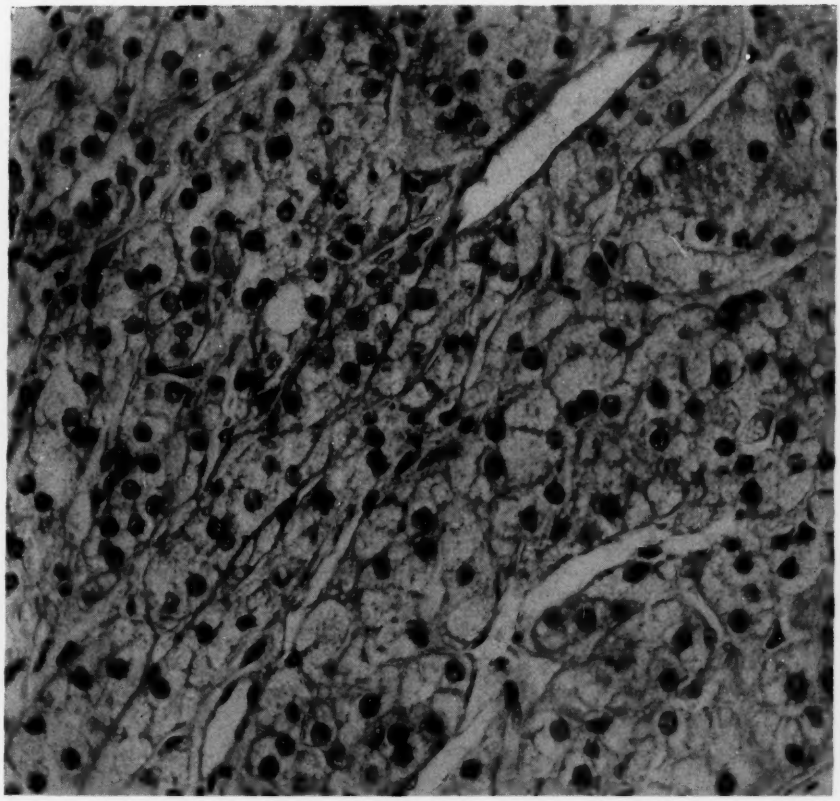

FIG. 2. Microscopic section of the tumor removed from Patient M. G., showing polygonal cells with clear vesicular cytoplasm and small round nuclei. In some areas the cells had finely eosinophilic cytoplasm. (×430.)

Paper chromatography was first carried out in toluene:ethylene-glycol for five days. The zone more polar than tetrahydrocortisol was eluted and rechromatographed in chloroform:formamide for seventeen hours. The area corresponding to 6β-hydroxycortisol was eluted, acetylated with acetic anhydride and pyridine at room temperature, and finally chromatographed on paper in the toluene:propylene-glycol system for seventeen hours. Chromatography of the acetate on silica-gel columns preceded infrared spectrometry and determination of the absorption spectrum in sulfuric acid.

Identification of the isolated 6β-hydroxycortisol was based on the properties as listed in Table 1. Infrared spectrometry was performed through the courtesy of Dr. T. F. Gallagher at the Sloan Kettering Institute and the results were consistent with the spectrum presented by the reference 6β-hydroxycortisol. Quantitation of the alpha-ketolic steroid content of the eluted zones of the paper chromatograms was made by the blue-tetrazolium reduction method previously reported (7). The values listed in Table 2 were obtained with reference to cortisol as unity.

TABLE 1. PROPERTIES OF 6β-HYDROXYCORTISOL

	Isolated	Reference*
1. Ultraviolet absorption in alcohol	241 mμ	241 mμ
2. Reaction with blue tetrazolium	+	+
3. Mobility (R_{THF})† in toluene: ethylene-glycol	0.15	0.13
4. Mobility (R_{THF})† in chloroform:formamide	0.15	0.15
5. Mobility diacetate (R_{DOC})‡ in toluene:propylene-glycol	0.10	0.10
6. Excitation and fluorescence maxima in sulfuric acid	460–510 mμ	460–510 mμ
7. Transient red color in sulfuric acid	+	+
8. Absorption spectrum in sulfuric acid (acetate) (maxima)	240, 275, 335, 390, 475 mμ	240, 270, 340, 390, 475 mμ
9. Soda fluorescence	+	+
10. Infrared spectrometry	Confirmed the identification (see text)	

* The 6β-hydroxycortisol reference was kindly supplied by Dr. Seymour Bernstein, Lederle Laboratories, Pearl River, New York.
† Mobility with respect to tetrahydrocortisol taken as unity.
‡ Mobility with respect to 11-desoxycorticosterone taken as unity.

RESULTS

In Table 2 are listed the amounts of 6β-hydroxycortisol and the major alpha-ketolic steroids found in the urines of the 3 patients. For comparison, normal values obtained by the present method are included. The amounts of tetrahydrocortisol (THF), tetrahydrocortisone (THE), and cortisol (F) are also tabulated for comparative purposes.

TABLE 2. URINARY STEROID EXCRETION (MG. PER 24 HOURS)

Subject	6-OH-F*	THF*	THE*	F*	17-KS†	17-OH-CS‡
6 Normal subjects—range —avge.	0.06–0.17 0.10	0.70–3.2 2.0	1.5–3.5 3.0	0.01–0.05 0.04	5–15	2–10
J.K. (normal) (ACTH)	0.17 0.24	0.76 2.7	1.8 5.1	0.05 0.77	N.D.§ N.D.	N.D. N.D.
W.K. (adrenal hyperplasia)	3.1	5.5	3.7	2.1	41.0	34.0
L.W. (adrenal hyperplasia)	1.8	17.0	8.6	7.8	95; 151	123.0
M.G. (adrenal adenoma) (ACTH)	0.6 1.2	1.4 2.9	1.2 2.2	0.05 0.5	5.6 9.7	2.8 11.2

* 6-OH-F = 6β-hydroxycortisol; THF = tetrahydrocortisol; THE = tetrahydrocortisone plus 3α-allotetrahydrocortisol; F = cortisol.
† 17-KS = routine 17-ketosteroid values obtained by the Callow procedure.
‡ 17-OH-CS = routine 17-hydroxycorticosteroid values obtained by the Silber-Porter procedure.
§ N.D. = not determined.

In agreement with the report of Nadel *et al.* (5), small amounts of 6β-hydroxycortisol were found in the urine of normal subjects. Katz *et al.* (6), using a different extraction procedure, found a range of 181–495 μg. per day in the urine of normal subjects. The excretions of THF, THE and F in the 2 patients with adrenocortical hyperplasia were greatly elevated, as is usually the case. The excretion of F in some patients with adrenocortical hyperfunction may indicate an overload of the system involved in the reduction of the ring-A alpha-beta unsaturated ketone. Patients L.W. and W.K. also excreted large amounts of 6β-hydroxycortisol. The implication is discussed later.

Patient M.G. presented a normal urinary alpha-ketolic steroid pattern, with the exception of a pronounced increase in 6β-hydroxycortisol. The intravenous administration of ACTH resulted in an increased output of 6β-hydroxycortisol, and also of THF and THE.

DISCUSSION

The *in vivo* 6β-hydroxylation of steroids was first indicated by reports of the identification of 6β-hydroxypregnanediols in urine by Lieberman *et al.* (8). Axelrod and Miller (9) demonstrated the presence of a 6β-hydroxylase in rat liver tissue. Hayano *et al.* (10) found it in the corpus luteum of cow ovaries, and Haines (11) reported that hog adrenal tissue contains the enzyme. 6β-Hydroxycortisol was isolated by the Worcester group from the urine of a patient with Cushing's disease to whom cortisol had been administered after an adrenalectomy. Later they reported the isolation of 1.1 mg. of 6β-hydroxycortisol from 50 liters of urine collected from normal men (5). Katz *et al.* (7) found that the substance is present in the nonconjugated form.

This report indicates that, in adrenal hyperfunction, 6β-hydroxycortisol may be a major urinary excretory product. In adrenal adenoma, as described in Patient M.G., the output of this substance was elevated while the output of the usual alpha-ketolic steroids appeared to be in the normal range. It is not possible at the present time to ascertain the source of the 6β-hydroxylated steroid, although the increase following administration of ACTH suggests adrenal origin. The possibility should also be considered that impairment of the pathway for metabolism of cortisol to tetrahydrocortisol may have caused a detour to 6β-hydroxylation, which resulted in a greater water solubility. At the present time one cannot distinguish between altered cortisol metabolism resulting in more urinary 6β-hydroxycortisol, and increased adrenocortical production. In this regard, Ulstrom *et al.* (12) found that 6β-hydroxylation appears to be a major pathway for the metabolism of cortisol in the newborn. It should be emphasized that

although 6β-hydroxycortisol produces a reaction in the Porter-Silber procedure, present extraction methods will not permit its quantitation since it is not completely removed from the urine by chloroform extraction.

In each of these cases, no ring-A reduced metabolites of 6β-hydroxycortisol were observed, although a search was made for them. Possibly the extraction procedure will not remove them from urine. No trace was found of blue-tetrazolium reducing substances more polar than 6β-hydroxycortisol.

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URINARY 17-KETOSTEROIDS FOLLOWING ADMINISTRATION OF LONG-ACTING TESTOSTERONE ESTERS

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ABSTRACT

In 29 apparently healthy subjects aged 19 to 23 years, with the same diet and physical exercise, urinary 17-ketosteroids (17-KS) were measured before and after a single intramuscular injection (in oil) of the equivalent of 200 mg. of testosterone, administered in the form of the p-cyclopentyl propionate (CPP) to 9 subjects, of the enanthate (ENT) to 10 subjects, and of the p-hexoxyphenyl propionate (HPP) to 10 subjects. The average pretreatment 24-hour excretion in the 29 subjects was 10.6 mg. of dehydro*epi*androsterone equivalent with 95 per cent fiducial limits at 5.5 and 15.7 mg. From a complete analysis of variance of pretreatment excretion the 95 per cent fiducial limits of the "normal" day-to-day variation were assessed as 77 and 124 per cent. Administration of the different testosterone esters resulted in an increased 17-KS excretion of varying degree and duration; the effect of CPP and ENT lasted at least five but less than eight days, whereas that of HPP lasted at least eight but less than eleven days. When compared with the pretreatment levels, the period of increased 17-KS excretion was followed by a transitory but signifcant reduction in the CPP-treated group, by a probable but not certain reduction in the ENT-treated group, and by unchanged urinary 17-KS levels in the HPP-treated group. The excretion of 17-KS was significantly greater following the administration of CPP and ENT than following the injection of HPP; the average "conversion rate" ($\pm$standard deviation of a single estimate) calculated from the excretion data of 7 out of the first 9 postinjection days was 20.1 ± 11.0 per cent for CPP, 16.9 ± 8.1 per cent for ENT, and 4.8 ± 5.9 per cent for HPP. The large individual variations in the "conversion rate" of esterified testosterone suggest similarly large variations in other steroid conversion reactions in the human. Therefore caution is needed in the interpretation of quantitative data obtained in steroid metabolic studies carried out on a limited number of subjects.

ASSESSMENT of the therapeutic value of long-acting testosterone preparations is still an incompletely solved problem. Methods of evaluation generally include *a*) measurement of the weight of the accessory reproductive organs in castrated male rodents at different intervals following a single injection of the compound, *b*) a study of the classic clinical

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effects (for instance, in the treatment of hypogenitalism), and *c*) estimation of urinary 17-ketosteroid (17-KS) excretion in subjects treated with the compound in question. As pointed out by Hamburger (1), each of these methods has its limitations. Species differences may sometimes render it unsafe to apply conclusions drawn from rodent experiments to the conditions in the human being. Clinically, it is difficult to evaluate the effects of the long-acting steroid objectively and quantitatively. The value of 17-KS assays is limited by the extent of the individual variations in the "conversion rate"[1] of long-acting testosterone preparations. The magnitude of this variation is not known at present. Moreover when urinary 17-KS assays are used as a possible parameter for the duration of action of these testosterone esters, it is assumed—implicitly or explicitly—that hydrolysis of such esters in the organism is a prerequisite for their therapeutic effectiveness and that no major physiologic activity can be expected from such preparations beyond the period of elevated 17-KS excretion. For the time being, both these assumptions seem to lack conclusive evidence.

Recently, a new long-acting testosterone ester, the p-hexoxyphenyl propionate (HPP) has been described (2, 3). The duration and intensity of the androgenic effect of this ester in castrated rats greatly exceeded that of similar esters, such as the enanthanate (ENT) or the β-cyclopentyl propionate (CPP). Therefore it seemed pertinent to compare the effect of these 3 esters on urinary 17-KS excretion in a group of healthy young volunteers.

MATERIAL AND METHODS

Subjects. A group of 29 apparently healthy young soldiers, aged 19 to 23 years, volunteered for this study. They were members of the same combat unit, and received the same diet and carried out the same physical exercise. Their allocation to the different treatment groups was carried out with the aid of a table of random numbers (4).

Material. The 3 testosterone esters used in this study were the β-cyclopentyl propionate (5), the enanthate (6), and the p-hexoxyphenyl propionate (2, 3). They were administered intramuscularly in oily solution.

Method of assay. Urinary 17-ketosteroids were assayed by the Vestergaard (7) micromodification of the Zimmermann-Callow (8) technique. Values are expressed in dehyro*epi*androsterone equivalents per twenty-hour hours. The slight modifications introduced by us and the data on the over-all reliability of the method in our hands have been presented in a previous paper (9). All analyses were carried out in quadruplicate, *i.e.*, in duplicate by two technicians. In a few cases in which the deviation between the mean values obtained by the two technicians was more than 10 per cent, the assays were repeated.

[1] This expression will be used in this communication in a loose sense to denote the increase in urinary 17-ketosteroid excretion above pretreatment levels. It is realized that this value is a result of the inhibition of endogenous 17-KS excretion on the one hand, and the increase due to the conversion of exogenous testosterone on the other. Thus the actual conversion may be somewhat more than calculated from the excretion data.

Plan of study. Due to the training program of this group of soldier volunteers, urine specimens could only be collected on seventeen out of twenty-four days of the study. The plan of study, and also the main results, are shown in Tables 1 to 3. It would appear from the data in these tables that 4 pretreatment urine specimens were collected from each volunteer during a period of five days. On the sixth day, each subject was given an intramuscular injection of the equivalent of 200 mg. of testosterone in the form of one of the 3 esters studied—CPP being given to 9 subjects, ENT to 10, and HPP to 10. Urine was collected on the subsequent five days (first postinjection period), after which the collection had to be discontinued for two days. Urine was then collected for two days (second postinjection period), followed by an intermission of one day. Urine was collected again for two days (third postinjection period), followed by an intermission of two days. Then two days of collection (fourth postinjection period) were followed by one day on which no urine could be collected. The study was finished with a two-day collection of urine (fifth postinjection period). One missing entry (Table 2) was replaced by calculation, using conventional methods (10). Thus the data comprised 492 analyses carried out in quadruplicate on urine specimens collected from 29 volunteers on seventeen days.

RESULTS

The original 492 excretion values are shown in Tables 1 to 3.

Before the influence of the different testosterone esters is examined, a fair amount of information can be obtained from the analysis of the pretreatment excretion data, combined for all the 29 subjects. The analysis of variance shown in Table 4 indicates that none of the differences between the groups was significant, *i.e.*, random allocation of the subjects to the different treatment groups was not contradicted by the data.

From the 115 pretreatment values, a mean 17-KS excretion of 10.6 mg. per twenty-four hours was calculated. Following elimination of the variation dependent upon individuals (but not that dependent upon different days), a standard deviation (S.D.) of 1.22 mg. per twenty-four hours was calculated. Multiplication of this value by the tabulated value of t (with 86 d.f.) yielded 95 per cent fiducial limits at 8.2 and 13.1 mg. per twenty-four hours, or when expressed as a percentage of the mean value, at 77 and 124 per cent. Thus the day-to-day variation in an individual under fairly constant circumstances (for instance during hospitalization) can be expected in 19 out of 20 cases not to exceed these limits, provided the completeness of urine collection is secured by adequate measures.

From the means of the pretreatment values in the 29 cases it was also possible to calculate fiducial limits within which 95 per cent of subjects of the same age bracket and racial background could be expected to fall. These limits were 5.5 and 15.7 mg. per twenty-four hours (with a mean of 10.6 mg.) when a normal distribution of individual values was assumed, and 6.7 and 16.1 mg. per twenty-four hours (with a mean of 10.4 mg.) when a lognormal distribution (11) was assumed.

An analysis of variance, similar to that shown in Table 4 was applied to

TABLE 1. URINARY 17-KETOSTEROID EXCRETION (MG./24 HOURS) IN A GROUP OF 9 MALE SUBJECTS BEFORE AND AFTER A SINGLE INTRAMUSCULAR INJECTION OF THE EQUIVALENT OF 200 MG. OF TESTOSTERONE, ADMINISTERED AS THE β-CYCLOPENTYL PROPIONIC ACID ESTER

Subject		Control period (pretreatment values)				First postinjection period					Second post-injection period		Third post-injection period		Fourth post-injection period		Fifth post-injection period	
		Day of study																
Initials	Age	1	2	4	5	6	7	8	9	10	13	14	16	17	20	21	23	24
Å-N	20	15.9	11.8	11.1	9.8	22.8	48.2	23.9	20.9	16.0	14.1	11.2	9.2	7.8	9.8	13.3	7.6	9.4
N-S	20	10.2	12.2	9.6	9.9	19.1	21.0	22.1	17.8	13.2	11.8	9.9	6.7	6.6	6.0	9.4	6.9	8.0
S-L	20	11.6	11.8	12.4	10.7	12.0	11.4	11.6	14.7	11.2	7.7	10.8	10.6	7.5	5.4	7.8	7.4	9.6
Å-D	20	7.0	6.8	7.1	7.6	13.8	20.7	17.0	10.8	11.2	9.6	9.6	5.7	7.2	5.8	7.8	7.0	8.6
R-C	20	9.6	8.8	10.0	8.4	22.4	23.2	17.6	14.4	10.6	9.1	9.2	9.2	6.6	12.2	11.4	10.0	8.9
W-L	20	8.4	7.5	9.6	9.7	20.6	16.6	16.0	24.4	11.0	9.0	8.0	7.4	5.3	5.4	8.8	7.2	6.8
B-E	21	8.2	10.1	11.8	9.3	24.1	26.1	21.2	20.6	14.6	6.3	10.0	8.7	7.0	8.4	14.3	8.8	7.6
W-M	21	15.0	15.6	15.0	14.8	19.5	20.1	18.1	19.4	19.0	12.8	10.8	13.0	8.6	7.5	12.6	12.0	12.4
K-N	19	11.8	11.9	11.8	12.0	24.0	22.8	26.6	16.8	13.6	15.8	16.4	12.5	12.7	16.0	15.2	10.9	9.8

TABLE 2. URINARY 17-KETOSTEROID EXCRETION (MG./24 HOURS) IN A GROUP OF 10 MALE SUBJECTS BEFORE AND AFTER A SINGLE INTRAMUSCULAR INJECTION OF THE EQUIVALENT OF 200 MG. OF TESTOSTERONE, ADMINISTERED AS THE ENANTHATE

Subject		Control period (pretreatment values)				First postinjection period					Second post-injection period		Third post-injection period		Fourth post-injection period		Fifth post-injection period	
		Day of study																
Initials	Age	1	2	4	5	6	7	8	9	10	13	14	16	17	20	21	23	24
F-S	21	9.5	10.6	10.1	10.0	14.2	16.8	17.0	18.8	11.0	10.8	7.6	7.3	7.5	5.6	7.0	5.7	6.4
S-M	19	11.4	9.2	8.9	8.6	15.6	15.1	17.0	15.8	12.0	10.8	9.8	7.1	6.6	5.7	5.8	6.2	6.9
J-N	19	8.8	8.3	12.4	11.6	17.2	16.2	20.8	20.8	12.6	15.4	13.2	9.7	11.4	9.4	11.4	10.7	10.7
R-L	19	8.8	10.4	11.8	10.2	27.5	38.1	19.2	16.3	17.0	12.4	8.5	8.9	10.0	10.3	12.0	9.1	11.2
B-M	19	7.0	7.5	8.8	8.0	10.8	15.2	12.6	10.9	8.2	5.2	4.6	4.4	12.1	6.0	5.3	11.8	7.5
H-N	20	12.6	11.9	12.0	12.6	21.2	35.4	18.7	18.2	19.6	10.6	10.1	14.9	11.2	12.0	13.6	12.6	9.6
W-T	20	7.2	7.8	11.0	6.9	10.0	9.0	14.4	11.4	10.9	9.8	10.7	5.6	7.7	5.6	6.7	5.2	9.2
B-G	19	9.4	(10.2)*	11.2	11.6	22.0	16.6	15.7	10.1	11.8	15.1	13.6	14.2	14.1	9.1	10.8	9.8	10.5
P-N	20	8.1	7.6	8.6	9.1	14.0	15.4	14.5	16.0	10.2	9.2	7.8	8.0	8.2	7.2	8.2	6.0	6.5
H-N	20	10.5	10.6	11.6	11.0	14.1	19.0	18.1	18.6	11.2	9.7	9.0	6.9	5.3	6.7	5.4	7.4	11.0

* Missing entry calculated by the use of the general formula $y = rR + cC - G/(r-1)(c-1)$, where y is the missing item, r the number of rows, R the sum of values in the row with the missing value, c the number of columns, C the sum of the values in the column with the missing entry, and G the grand total, *i.e.*, the sum of all values.

TABLE 3. URINARY 17-KETOSTEROID EXCRETION (MG./24 HOURS) IN A GROUP OF 10 MALE SUBJECTS BEFORE AND AFTER A SINGLE INTRAMUSCULAR INJECTION OF THE EQUIVALENT OF 200 MG. OF TESTOSTERONE, ADMINISTERED AS THE P-HEXOPHENYL PROPIONIC ACID ESTER.

Subject		Control period (pretreatment values)				First postinjection period					Second post-injection period		Third post-injection period		Fourth post-injection period		Fifth post-injection period	
		Day of study																
Initials	Age	1	2	4	5	6	7	8	9	10	13	14	16	17	20	21	23	24
U-N	19	9.6	11.0	11.8	11.4	8.4	10.4	9.5	9.4	7.8	8.7	9.7	10.3	7.8	10.0	9.2	8.0	7.4
S-G	19	9.1	7.8	10.6	8.0	9.4	11.4	10.4	11.8	10.8	10.4	9.6	8.2	7.7	8.0	8.4	7.7	9.0
A-Y	20	11.4	10.3	10.4	10.9	12.3	12.8	11.8	14.0	11.4	12.4	10.2	12.5	9.2	8.6	10.6	8.8	6.7
L-M	20	8.8	11.2	10.2	10.1	8.4	8.5	7.8	10.6	9.0	10.9	13.1	8.9	8.6	8.4	9.8	8.6	9.8
D-N	19	16.3	15.8	17.6	16.6	18.5	20.3	20.4	23.4	20.5	18.6	19.4	16.2	16.4	17.8	18.9	14.3	13.6
Q-T	19	10.4	12.3	11.6	12.2	8.0	11.6	11.2	12.5	13.5	13.4	13.0	11.0	12.2	10.2	11.4	13.2	11.1
J-N	20	9.6	7.2	9.0	12.6	11.8	12.2	13.4	14.2	13.4	13.2	13.0	12.8	10.1	9.8	10.4	10.4	10.6
A-N	23	17.0	19.0	20.2	17.2	19.2	19.5	18.6	19.1	18.6	21.6	19.3	14.8	16.0	14.2	17.3	13.7	13.8
S-N	19	7.8	8.8	8.5	8.9	10.1	10.8	10.0	10.4	10.4	12.2	11.4	9.5	9.2	9.2	9.8	7.2	9.6
L-G	20	8.2	9.8	6.4	10.7	12.0	13.0	11.4	9.6	8.3	11.5	11.6	9.0	8.4	6.5	13.6	6.1	5.8

TABLE 4. ANALYSIS OF VARIANCE FOR THE PRETREATMENT (CONTROL) VALUES IN 29 MALE VOLUNTEERS
(combined data from Tables 1 to 3)

Source of variation	Degrees of freedom	Sum of squares	Mean square	F.
Group III *vs.* I+II	1	35.75	35.75	1.43
Group I *vs.* II	1	13.81	13.81	<1.0
Between treatment-groups	2	49.56	24.78	<1.0
Between subjects within groups	26	650.64	25.02	
Between subjects	28	700.20	25.01	17.49***
Between days	3	9.34	3.11	2.17
Residual error	83	118.78	1.43	
Total	114	828.32		

*** $P < 0.001$.

the data of Tables 1 to 3. In this way it was possible to compare the pretreatment values in Table 3 with those for the first 7 specimens following the administration of the testosterone ester. This analysis is shown in Table 5, and clearly indicates that the subjects in this group excreted more 17-KS following treatment with HPP than in the pretreatment period. The difference was highly significant ($P < 0.001$).

The duration (in days) of elevated 17-KS excretion following the injection of the 3 esters was assessed by calculating the individual differences (in mg. per twenty-four hours) between the pretreatment value and the postinjection values obtained during the various postinjection periods. Upon dividing the mean difference by its standard error, it was possible to determine whether or not this ratio significantly exceeded the corresponding tabulated *t*-value, *i.e.*, whether or not the difference was statistically

TABLE 5. ANALYSIS OF VARIANCE FOR THE DATA OF TABLE 1
(only the data of the first 7 treatment days are included)

Source of variation	Degrees of freedom	Sum of squares	Mean square	F.
Control days *vs.* treatment days	1	48.35	48.35	15.9***
Between days within groups	9	27.38	3.04	
Between days	10	75.73	7.57	
Between subjects	9	1285.38	142.82	
Residual error	90	191.93	2.13	
Total	109	1553.04		

*** $P < 0.001$.

significant. For instance, when the mean of the second postinjection period was compared with the mean pretreatment value, the calculation indicated a significant difference for the values in Table 3 ($t=3.80$) but not for those in Table 1 ($t=0.02$) or Table 2 ($t=0.41$). Thus the duration of increased 17-KS excretion was at least five, but less than eight days following the administration of CPP or ENT, whereas it was more than eight but less than eleven days in HPP-treated subjects. Consequently, the duration of increased 17-KS excretion in subjects treated with HPP exceeded significantly the duration in those treated with CPP or ENT. The period of increased 17-KS excretion was followed by a transient but significant decrease in the CPP group, a probable but not certain decrease in the ENT group, and by unchanged urinary KS-levels in the HPP group.

Another parameter is the amount of 17-KS excreted following the injection of a standardized dose of the testosterone ester in question. This was

TABLE 6. "CONVERSION" OF DIFFERENT TESTOSTERONE ESTERS INTO URINARY 17-KETOSTEROIDS EXPRESSED AS A PERCENTAGE OF THE ADMINISTERED AMOUNT OF TESTOSTERONE

Testosterone ester tested	5 Days	7 Days
	Mean conversion ±S.D.*	Mean conversion ±S.D.*
p-Hexoxyphenyl propionate	3.1±4.8	4.8± 5.9
β-Cyclopentyl propionate	20.2±9.8	20.1±11.0
Enanthate	16.4±7.8	16.9± 8.1

* Standard deviation of a single estimate.

calculated from the data of Tables 1 to 3 for each subject, by summation of the excretion values for a five-day (or a seven-day) postinjection period and by subtracting from this value five (or seven) times the mean pretreatment value for the subject in question. The mean values for the various groups (with the standard deviation of an individual estimate), expressed as a percentage of the administered dose, are shown in Table 6. From these data it appears that there is a marked difference between CPP and ENT on the one hand, and HPP on the other hand. Only 3 per cent of the administered dose was excreted during the first postinjection period in the HPP group, whereas 20 and 16 per cent, respectively, of the administered dose was excreted in the CPP and ENT groups. The difference was statistically highly significant ($t=4.19$ with 27 d.f.). When the values of the first and second postinjection period were combined, the "percentage conversion" figures remained virtually unchanged as far as the CPP (20 per cent) and ENT (17 per cent) groups were concerned, whereas the value in the HPP group increased to 5 per cent. Assuming that the excretion in the

HPP group was roughly the same during the two days between the first and second postinjection periods (when no urine could be collected), then the calculated over-all recovery was 6.5 per cent. This was still considerably less than the values obtained in the two other groups.

These conclusions are based on arithmetic mean values. However, the individual variation in the different groups was considerable; in fact, "conversion rates" in the CPP group varied between −1 and 36 per cent, in the ENT group between 6 and 33 per cent, and in the HPP group between −6 and 12 per cent. If standard deviations are calculated and these S.D.-values are multiplied with the corresponding *t*-values, fiducial limits can be calculated within which 95 per cent of individual "conversion rates" will fall. A calculation of such limits yielded surprising results, since in none of the groups was it possible to exclude 0 per cent as a plausible *individual* value. The limits were calculated as −2.8 and 42.8 per cent for CPP, −1.2 and 34.0 per cent for ENT, and −8.5 and 18.1 per cent for HPP. Repetition of the calculations, assuming a lognormal rather than a normal distribution of individual "recovery rates," increased rather than decreased the foregoing variation. It is of interest that no correlation was found between pretreatment values and "percentage conversion." It is realized that a final clarification of this problem would require much more data obtained on much larger groups of subjects.

DISCUSSION

There are several conflicting reports on urinary 17-KS excretion following the administration of a certain type of long-acting testosterone ester. For instance, Schneider and Schuchter (12) found that the administration of 250 mg. of ENT increased 17-KS excretion for about twenty days, with a "conversion rate" of 56 to 62 per cent. Bauer (13) observed elevated 17-KS excretion lasting for approximately twenty-five days following the injection of 100 mg. of this ester, with a "conversion rate" of no less than 200 per cent. On the other hand, Schreus and Ruhrmann (14) and Ruhrmann (15) noted no increase in 17-KS excretion following the injection of 100 mg. of ENT, and only a questionable increase in 1 of the subjects given 250 mg. Hamburger (1), administering 390 mg. of this ester to 2 male subjects, found an increased excretion of 17-KS lasting for six to eight days and a "conversion rate" of 32 to 43 per cent. Our present data are in fairly good agreement with those of Hamburger (1) but not with those of the other authors (12–15).

Before attempting to interpret the seemingly discrepant findings of these various authors, it should be realized that there are many variables which might significantly influence the results obtained. The size of the dose administered may be critical; it is likely that huge doses of esterified testosterone are handled by the organism in a different manner than

relatively small doses. Furthermore the sex and especially the age of the subjects may be of importance. Another factor may be the general condition of the subject; relative inactivity in a hospital bed as compared to heavy physical exercise may influence endogenous 17-KS excretion as well as the "conversion rate" of testosterone to an extent which is as yet incompletely determined. Thus it has been recently demonstrated that exhaustive exercise in young soldiers will significantly decrease endogenous 17-KS excretion (16); whether such exercise will also influence the conversion of administered testosterone esters into 17-KS, is not known. Dietary factors, the general endocrine status (*e.g.*, thyroid activity), and certain pathologic conditions also exert an influence on the conversion of testosterone into urinary 17-KS.

The data of the present paper indicate that in addition to all these factors, the individual variation in a group of apparently healthy young men receiving the same diet and performing the same physical exercise may be much greater than previously thought. During a period of five days after the administration of ENT, the "conversion rate" in 10 subjects varied between 7 and 33 per cent, whereas in 9 others treated with CPP the individual "conversion rate" varied between 1 and 36 per cent. Therefore, in the absence of a statistically valid estimate of variability, it does not seem justifiable to make quantitative generalizations on the basis of data obtained on 2 or 3 subjects. A perusal of the available literature indicates that our present concepts of steroid conversion reactions in the human are more often than not based on very small series of observations. It might be useful, therefore, to bear in mind the degree of uncertainty involved in some of our present steroid metabolic concepts.

It is also an open question how the "ideal" testosterone ester should behave in the human organism. If we assume that approximately 4 to 5 mg. of testosterone should be made available to the organism daily, and if we also assume an over-all "conversion rate" of let us say 20 per cent, then one would expect that an adequate liberation of testosterone would be reflected in a urinary 17-KS increase of approximately 1 mg. per twenty-four hours. This type of assessment is further complicated by the simultaneous depression of the endogenous excretion of 17-KS and by the fact that it has not been established that any therapeutic effects of the administered esters can be expected beyond the period of increased 17-KS excretion. If this were the case, then the point raised by Hamburger (1) is relevant, namely, that from the therapeutic point of view it might be an advantage to use esterified testosterone preparations with a low "percentage recovery."

The generally low over-all recoveries raise once more the problem of the metabolic fate of esterified testosterone in the human organism. Are such esters retained for a longer time, and if so, where? What happens to the

bulk of the esterified testosterone? Clinical studies in which esters of labeled testosterone are administered may be informative in this respect, and will be awaited with interest.

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THE ESTIMATION OF EPINEPHRINE AND NOREPINEPHRINE IN HUMAN PLASMA*

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ABSTRACT

A modification of the ethylenediamine technic for the measurement of plasma epinephrine and norepinephrine is described, and the results of studies in normal subjects and patients are reported. Normal subjects had higher norepinephrine levels during mild activity than at bed rest, and an increase in both epinephrine and norepinephrine levels was observed after strenuous exercise. The plasma concentration of epinephrine was elevated during hypoglycemia following the administration of insulin to a normal subject. The concentrations of plasma amines in patients with essential hypertension, diabetes, adrenal insufficiency or hypopituitarism were not significantly altered. However, an elevation of epinephrine level was observed in patients with hyperthyroidism. The reresults of studies performed in 9 patients with pheochromocytoma confirmed the usefulness of plasma amine determinations in the diagnosis of this disorder, and showed a high correlation between the proportions of epinephrine and norepinephrine in plasma and tumor. Increased concentrations of plasma amines following injection of histamine were observed in patients with paroxysmal hypertension due to pheochromocytoma, but not in patients with essential hypertension. The ethylenediamine method appears most useful for the detection of elevations in the plasma epinephrine and norepinephrine levels in patients with pheochromocytoma and for the detection, by repeated sampling, of changes occurring over short periods of time in a specific subject.

A VARIETY of physical-chemical procedures have been devised for detecting the small quantities of epinephrine and norepinephrine present in plasma. Most of the methods are adaptations and modifications of the trihydroxyindole procedure developed by Lund (1) or the ethylenediamine technic developed by Weil-Malherbe and Bone (2, 3). The results obtained with both types of procedure have been compared in several

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studies (4–6), and the advantages and limitations of each have been extensively discussed (6–10).

The following report describes a modification of the ethylenediamine technic used for the estimation of epinephrine and norepinephrine in adrenal venous and peripheral plasma (6, 11–15). Additional studies which contribute to an evaluation of the biologic validity of the results obtained by this method are also presented.

METHOD

Reagents and standards

Alumina (Woelm), nonalkaline (almost neutral), activity Grade I.

Ethylenediamine (95 per cent); redistilled. After distillation the ethylenediamine may be divided into convenient aliquots, frozen, and stored for as long as two months.

Ethylenediamine dihydrochloride, recrystallized from ethanol-water (60:40).

Acetic acid, reagent grade. A 0.33 *M* solution of acetic acid is prepared by adding 5 Gm. per liter of acid, shaking, and filtering.

Isobutanol, reagent grade; redistilled.

Sodium chloride, reagent grade.

Stock solutions of the standards are prepared by dissolving epinephrine and norpinephrine bitartrate[1] in 0.33 *M* acetic acid to give a concentration of 50 μg. per liter of free base. Working standards are prepared immediately before use by diluting stock solutions with cold distilled water.

Procedure

Peripheral blood is drawn into a clean syringe and discharged into a tube containing 3 drops of heparin. Adrenal venous blood is collected directly into a tube containing heparin. The plasma is separated immediately by centrifugation and stored at freezing temperatures until extracted.

Extraction is performed by adding 0.3 Gm. of alumina to 5 ml. of plasma in a glass-stoppered 12-ml. centrifuge tube. The tube is shaken for three minutes and then centrifuged for thirty seconds; the supernatant plasma is discarded, and 5 ml. of cold distilled water is added. The tube is shaken for ten to twenty seconds, centrifuged for thirty seconds, the water aspirated, and the washing repeated. Three ml. of 0.33 *M* acetic acid is added and the tube is shaken for three minutes. After centrifugation, the supernatant liquid is transferred to a glass-stoppered centrifuge tube. The elution is repeated with 2 ml. of acetic acid and the eluates are combined. The eluates are stable and may be frozen and stored. To determine the recovery from plasma, additional samples containing known amounts of epinephrine and norepinephrine (0.2 μg. per 5 ml.) are prepared and carried through the foregoing procedure. Duplicate standards and reagent blanks are carried through the procedure with each group of unknowns.

The rest of the procedure is carried out in a dark room illuminated only by ruby or orange tungsten lamps. Ethylenediamine and ethylenediamine dihydrochloride, 7 parts to 5, are mixed immediately before use; 1.2 ml. of this solution is added to each tube and thoroughly mixed. The tubes are then incubated for forty minutes at 50°C. After cooling to room temperature, the solution is saturated with sodium chloride, and 4 ml. of isobutanol is added. The tubes are then shaken for three minutes and centrifuged.

[1] Obtained through the courtesy of the Sterling-Winthrop Research Institute, Rensselaer, New York.

Measurement of fluorescence

The fluorescence of the isobutanol extracts is measured with a Farrand photoelectric fluorometer, using a Baird interference filter (436 mμ), 2 glass filters (Corning set 5–74), and 2 neutral density filters (Kodak wrattan Gelatin N.D., .30) to provide an excitation wave length of 436 mμ. The first readings are made with secondary filters which have a maximum transmission of 485 mμ (Corning set 4–104) after adjustment of the instrument so that 0.2 μg. of norepinephrine produces a 1/2 to 2/3 scale deflection. The samples are then read with a red filter (Corning 2-62) after the instrument is readjusted so that 0.2 μg. of epinephrine causes a 1/2 to 2/3 scale deflection. The reagent blank is equivalent to approximately 0.01 μg. of catechol amine. The results are calculated by the following formula:

$$N = (b - ny)\ (m/m - n)$$
$$E = y - N/m$$

where

N = the concentration of norepinephrine,
E = the concentration of epinephrine,
b = the amount of norepinephrine present if the galvanometer deflection at 485 mμ is entirely due to norepinephrine,
y = the amount of epinephrine present if the reading with the red filter is entirely due to epinephrine,
n = the ratio of fluorescence of the standards E/N at 485 mμ, and
m = the ratio of fluorescence of the standards E/N with the red filter.

The application of the method to adrenal venous plasma, which usually has a higher concentration of norepinephrine and epinephrine than peripheral plasma, requires smaller samples. The technic may be applied without further modification to as little as 2 ml. of plasma. However, when smaller quantities are analyzed, the sample may be diluted to a volume of 2 ml. with veronal buffer ($\frac{1}{6}M$) at pH 8.4. Also, standards may be adjusted to a more convenient concentration.

RESULTS

Laboratory Studies

Fluorescence spectra

The activation and emission spectra were established by readings of the epinephrine and norepinephrine standards at wave-length intervals of 5 mμ on the Aminco-Bowman spectrophotofluorometer (Fig. 1). Samples contained the equivalent of 12 μg. of epinephrine or norepinephrine per ml. in order to minimize the blank fluorescence. Similar peaks are obtained when smaller concentrations are used. The intensity of fluorescence of the standards varies directly with the concentration of epinephrine or norepinephrine in a range of 0.005 to more than 0.5 μg. per sample. Amounts as small as 0.001 μg. can be detected, although with less accuracy. The slopes of the standard curves vary slightly from day to day. In 18 experiments, the ratio of E/N at 485 mm. was 0.20 ± 0.005 and the ratio with the red filter was 3.24 ± 0.20.

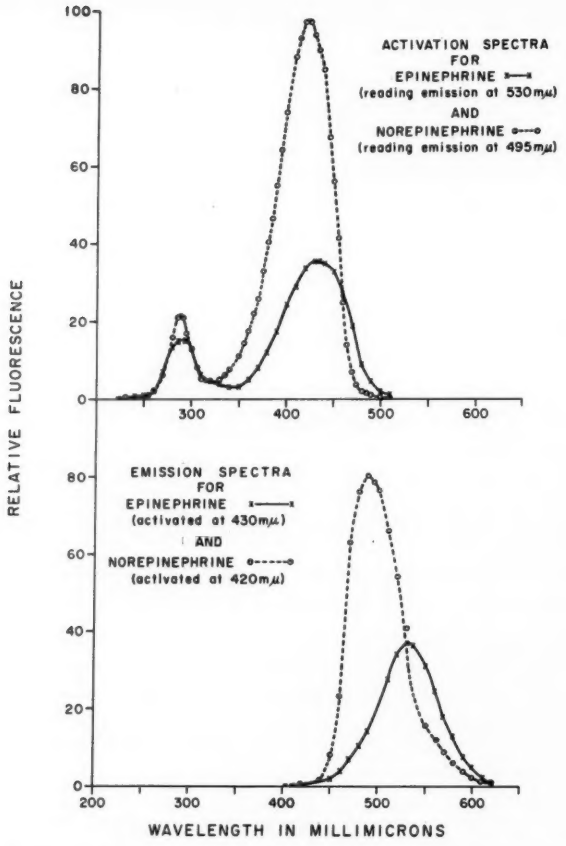

FIG. 1. Activation and emission spectra for epinephrine and norepinephrine, with reaction carried out in the darkroom.

Recovery experiments

In 27 consecutive experiments with the same lot of alumina, the recoveries for epinephrine were 99.4 per cent (S.D. ±4.07), and for norepinephrine 78.4 per cent (S.D. ±5.93). The recovery of smaller amounts of the amines was studied by determinations on 13 samples of plasma to which 0.2 to 4.8 millimicrograms of epinephrine and norepinephrine had been added (Table 1). The recoveries were satisfactory in those samples to which 0.6 millimicrogram or more had been added. The amine recovery for epinephrine was 97.8 ±7.81 per cent with a standard error of 11.3 per cent, and for norepinephrine 86.1 ±5.9 per cent with a standard error of 8.5 per cent. In duplicate determinations on 19 plasma samples to which 0.2 μg. of epineph-

TABLE 1. RECOVERIES OF SMALL AMOUNTS OF EPINEPHRINE AND NOREPINEPHRINE FROM PLASMA

Millimicrograms of epinephrine and norepinephrine added to plasma	% Recovered	
	Epinephrine	Norepinephrine
0.2	30	54
0.4	104	66
0.6	98	90
0.8	100	93
1.2	107	80
1.6	96	83
2.0	100	89
2.4	88	75
2.8	100	90
3.2	100	90
3.6	82	85
4.0	95	92
4.8	110	80

rine or norepinephrine had been added, the standard error was 3 per cent.

Studies were performed on the recovery of 6 related compounds from plasma. Five pairs of samples of each compound were prepared by the addition of 0.2 μg. of the substance to 5 ml. of plasma. Only dihydroxyphenylacetic acid and dopamine were found to increase the "apparent" concentrations of norepinephrine and epinephrine respectively (Table 2).

Epinephrine and norepinephrine disappear rapidly when dissolved in water and alkaline buffers, but are more stable in plasma. When 0.2 μg. of either amine was added to 5 ml. of plasma and incubated for nine hours at 37°C., the loss did not exceed 10 per cent per hour. The addition of buffered potassium cyanide (0.1 mg.) to the samples reduced recoveries 30 per cent but did not alter the rate of loss. This would indicate that plasma oxidases did not account for the decreases noted.

Clinical Studies

In the following studies, the application of the method to clinical conditions is illustrated. No correction for recoveries was made.

TABLE 2. "APPARENT" PLASMA CONTENT OF EPINEPHRINE AND NOREPINEPHRINE IN MILLIMICROGRAMS WHEN RELATED COMPOUNDS WERE ADDED TO PLASMA (0.2 μG. IN 5 ML.)

Compound	Millimicrograms of epinephrine	Millimicrograms of norepinephrine
Epinephrine	194	0
Norepinephrine	0	158
Dihydroxyphenylacetic acid	0	19
Dopamine	28	5
Metanephrine	1	1
Normetanephrine	2	0
3-Methoxy-4-hydroxymandelic acid	0	0
Dihydroxyphenylalanine	1	0

Normal subjects

Plasma was obtained from 22 laboratory workers, all free of disease, during routine activities. The mean epinephrine and norepinephrine concentrations are listed in Table 3. The absence of epinephrine in 14 of the 22 subjects and levels as high as 1.0 to 1.5 μg. per liter in a few, account for the large standard deviation. Since earlier studies (11) had shown that norepinephrine concentrations in peripheral plasma are related to muscular activity, blood specimens were also obtained from 12 subjects at rest after a standard basal metabolism test (Table 3). The basal metabolic rate of

TABLE 3. PLASMA EPINEPHRINE AND NOREPINEPHRINE CONCENTRATIONS IN NORMAL AND DISEASE STATES

Clinical state	No. of subjects	Epinephrine (μg./L.) mean ± S.D.	Norepinephrine (μg./L.) mean ± S.D.
Normal, active	22	0.51 ± 0.75	3.73 ± 1.35
Normal, basal	12	0.48 ± 0.13	1.55 ± 0.59
Essential hypertension	125	0.53 ± 0.53	3.42 ± 1.70
Hypertension and diabetes	14	0.80 ± 0.71	3.39 ± 1.62
Adrenal insufficiency	22	0.42 ± 0.28	3.50 ± 1.15
Hypopituitarism	9	0.38 ± 0.34	3.70 ± 1.35
Hyperthyroidism	10	1.08 ± 0.35	4.08 ± 1.44

these subjects varied from −15 to +16 per cent and no relationship was observed between the metabolic rate and plasma norepinephrine levels. The difference between norepinephrine concentrations at rest and those during normal activity was highly significant ($P < 0.001$).

The effect of strenuous exercise as compared to routine activity was investigated by obtaining blood specimens from a normal subject immediately before and two minutes after he had completed a three-mile run. The plasma epinephrine concentration increased from 0 to 3.5 μg. per liter, and the norepinephrine concentration from 2.9 to 8 μg. per liter.

Disease states

The results of determinations on peripheral plasma from patients with essential hypertension, hypertension and diabetes (receiving insulin), adrenal insufficiency (maintained with cortisone and desoxycorticosterone), hypopituitarism and hyperthyroidism are also listed in Table 3. In these patients, activity was not rigidly controlled. This fact may be important in explaining the variability of norepinephrine levels. Additional determinations were carried out on plasma from 2 patients with acromegaly, 4 with polycythemia, 4 with Cushing's syndrome and 2 with hyperaldosteronism. In all patients, concentrations of epinephrine and nor-

epinephrine in peripheral plasma were within normal limits with the exception of those with hyperthyroidism. In this latter group, the plasma epinephrine levels were significantly higher than those of the normal subjects ($P < 0.01$). In patients with renal disease characterized by marked elevations in the level of blood urea nitrogen, the spuriously elevated concentrations noted previously (6) were found. The "apparent" epinephrine values varied from 0.6 to 37.5 μg. per liter, and norepinephrine values varied from 5.3 to 13.0 μg. per liter in these patients.

Hypoglycemia

Previous studies of the effects of insulin-induced hypoglycemia in dogs and in psychiatric patients undergoing insulin-coma therapy have been reported (13, 14). The effects of 10 units of insulin administered intravenously to a 41-year-old normal subject are illustrated in Figure 2. The inverse relationship between blood sugar and plasma epinephrine levels is noteworthy. The small changes observed in the plasma norepinephrine levels do not appear to be related to the blood sugar concentrations.

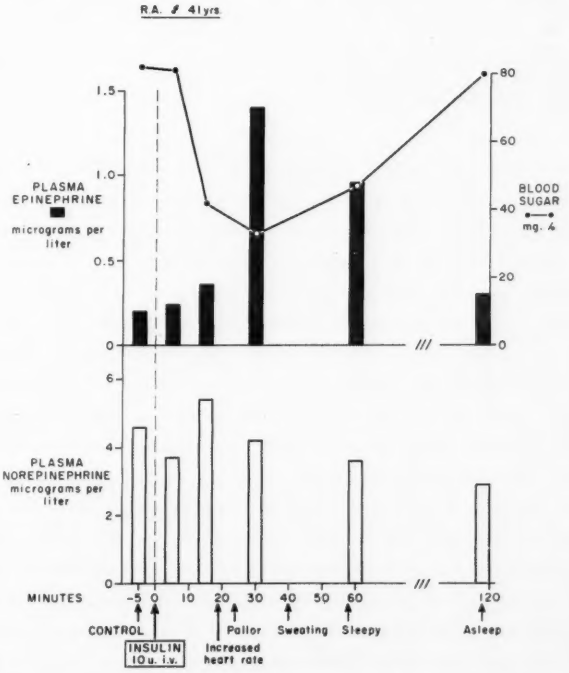

FIG. 2. Plasma epinephrine, norepinephrine, and blood sugar levels during insulin-induced hypoglycemia in a normal subject.

Pheochromocytoma

Plasma epinephrine and norepinephrine levels were measured in 9 patients with surgically verified pheochromocytomas. The plasma concentration and urinary excretion of the catechol amines before and after removal of the tumor, the response to phentolamine or histamine, and the tumor content of amines are recorded in Table 4. Figure 3 shows the close relationship between the plasma ratio of epinephrine and norepinephrine and the tumor content of these amines.

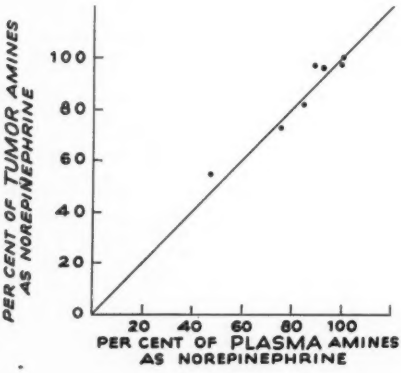

FIG. 3. The relationship between the percentage of norepinephrine in plasma and tumors removed from 7 patients with pheochromocytoma. The line is not drawn through the points but represents the line of identical distribution.

Two patients were studied before and after operation. Patient No. 1, a 17-year-old female, in whom polycythemia was diagnosed at age 3, was first noted to have an elevated blood pressure at the age of 16. Although she had no symptoms suggestive of paroxysmal discharge of an amine-secreting tumor, she did lose 20 pounds during the next year and became intolerant of heat. A diagnosis of pheochromocytoma was considered and plasma and urine catechol amine levels were measured (Table 4). At operation, 3 small extra-adrenal chromaffin tumors (1.5, 1.3 and 3.3 Gm.) were removed. Amine concentrations in plasma were determined before, during, and following the operation (Fig. 4) Although plasma levels of epinephrine and norepinephrine after surgery were consistently normal, hypertension persisted. The results of Regitine tests were positive on several occasions during this period. However, no response to histamine was noted. After two years, the patient was re-explored and a left nephrectomy carried out because of extensive infarction and necrosis of the kidney. The blood pressure then returned to normal levels, although no change in plasma catechol amine concentration was observed.

The other patient, No. 8, was a 49-year-old woman with an 18-month history of episodes of anxiety, palpitation and flushing. Her blood pressure

TABLE 4. HORMONAL STUDIES IN 9 PATIENTS WITH SURGICALLY VERIFIED PHEOCHROMOCYTOMA

Patient No.	Age & sex	Hypertension	Preoperative catechol amine levels*					Blood pressure response to		Postoperative catechol amine levels*					Tumor		
			Plasma (μg./L.)		Urine (μg./24 hrs.)					Plasma (μg./L.)		Urine (μg./24 hrs.)			Weight (Gm.)	Content (mg./Gm.)	
			N	E	N	E	T	Phentol-amine	Hista-mine	N	E	N	E	T		N	E
1	17 F	Persistent	10.7	0.7	800**	—		+		3.9	0.6			<100	6		
2	23 F	Persistent	19.8 17.8	0.0 1.5	2000	—		+						<100		100%†	—
3	12 M	Persistent	11.0	1.6			1000	+						<100	28	5.7	0.08
4	55 M	Persistent	11.4	6.2				+		4.0	0.0				‡		
5	39 M	Paroxysmal	3.1 Histamine→ 9.1	0.0 3.1			170		+	3.8 2.0	0.9 1.2			46	5	4.5	1.7
6	18 F	Persistent with superimposed paroxysmal	15.7 Paroxysm→ 20.6	0.5 0.1	703	12		+		3.1 3.1	1.0 1.0	32	3		30	3.29	0.06
7	22 M	Paroxysmal	3.3 Histamine→ 5.5	1.2 4.8			100		+	4.8	0.0			36	15	5.2	6.2
8	49 F	Paroxysmal	5.6 Paroxysm→ 5 mins. 98.0 later 13.2	0.0 0.0 2.6	261 521	30–69			+	9.0 8.7	0.5 0.6	68	10		29	3.97	0.70
9	17 F	Paroxysmal	6.6 Paroxysm→ 14.0	0.4 0.8	2000	63		+	+	2.9	0.8	105	2		90	2.35	—

* N = Norepinephrine; E = Epinephrine; T = Total catechols.
** This value was obtained by bio-assay. The remainder of the urinary catechols was determined by the method of Floding and Euler.
† Analysis by Dr. L. Aronow, recorded as percentage of total amines.
‡ A malignant tumor 10 cm. in diameter which involved the kidney.
Except in Patient 2, the tumors appeared to originate in the adrenal medulla.

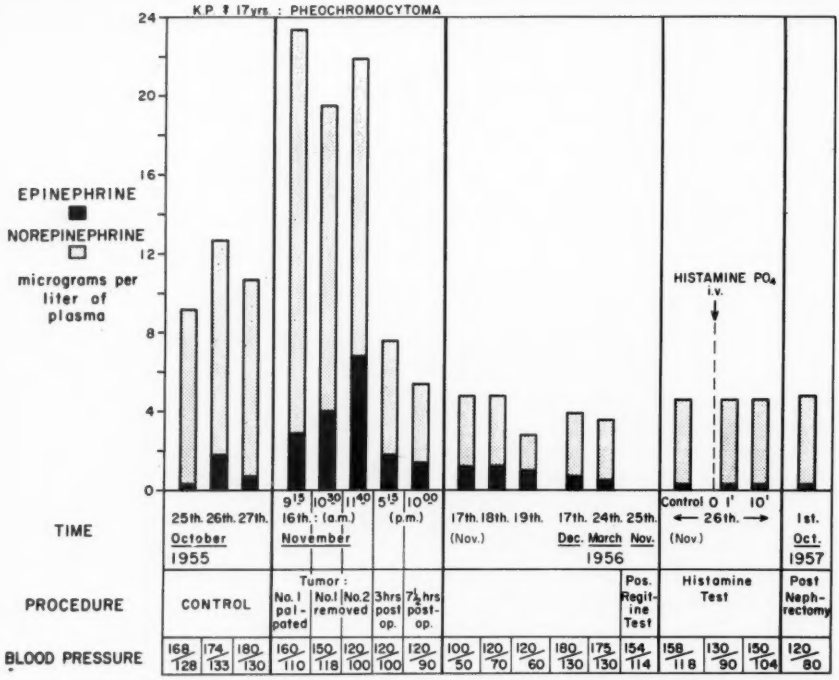

FIG. 4. Blood pressure and plasma amine levels in Patient 1 (Table 4) during a two-year period (October 1955 to October 1957).

was 130/80 mm.Hg between attacks, but was over 300/160 during paroxysms. Between attacks the plasma norepinephrine level was not elevated (Table 4), but at various stages during an attack the concentrations ranged from 15.2 to 98.0 μg. per liter. The presence of a mass in the region of the right adrenal was indicated by tomograms obtained after presacral oxygen insufflation. At operation a 29-gram tumor was found. The plasma epinephrine and norepinephrine concentrations before and after removal of this tumor are shown in Figure 5.

Histamine tests

The effects of injection of histamine on plasma amine levels in patients with paroxysmically secreting tumors have been described previously (12, 16) and these are illustrated by Patients 5, 6, 7 and 9 (Table 4). Histamine tests were also carried out on 28 subjects in whom a diagnosis of pheochromocytoma could not be established. In each case, a blood sample was obtained immediately preceding administration of 0.025 to 0.05 mg. of histamine base, and again three to four minutes after the injection. In all

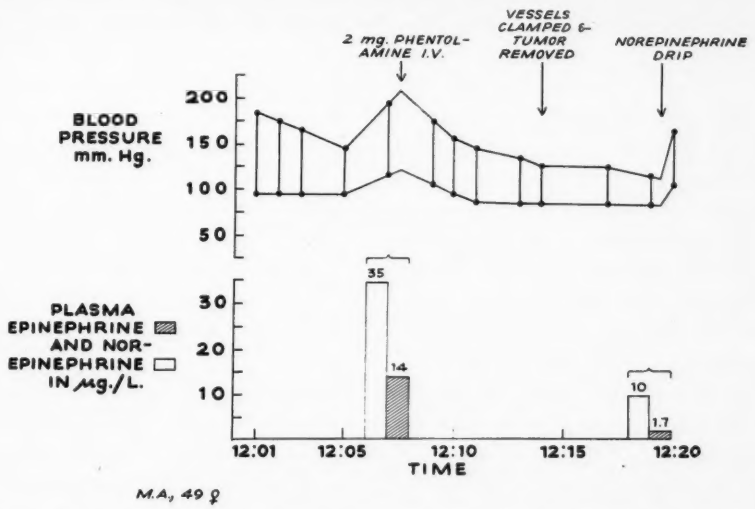

FIG. 5. Plasma epinephrine and norepinephrine concentrations and blood pressure at the time of removal of a 29-gram tumor, in Patient 8 (Table 4).

instances, the dose of histamine was sufficient to produce flushing, headache, and the characteristic fall in blood pressure. No significant change in plasma amine concentration was observed (Table 5). Exploration was also carried out in a patient in whom a moderate rise in blood pressure was observed following injection of histamine, but in whom no change was noted in the plasma epinephrine and norepinephrine levels. No tumor was found.

DISCUSSION

Certain aspects of the procedure for determinations of epinephrine and norepinephrine and its instrumentation deserve further comment. The activation and emission spectra of the ethylenediamine reaction products of epinephrine and norepinephrine as determined in the Aminco-Bowman spectrophotofluorometer are illustrated in Figure 1. A comparison of the emission spectra with those published by Persky and Roston (17) and Mangan and Mason (18) has been reported previously (19). The differences

TABLE 5. PLASMA EPINEPHRINE AND NOREPINEPHRINE LEVELS IN 28 HYPERTENSIVE SUBJECTS WITHOUT PHEOCHROMOCYTOMA BEFORE, AND 2–4 MINUTES AFTER THE INTRAVENOUS INJECTION OF 0.025 TO 0.05 MG. OF HISTAMINE BASE

	Epinephrine (μg./L.)	Norepinephrine (μg./L.)
Control	.6 ± .6	3.8 ± 2.2
After histamine	.5 ± .7	3.9 ± 2.2

in the three curves appear to be the result of exposing the fluorescent products of norepinephrine to daylight or other sources of light of short wave lengths. The shift noted in the peak of fluorescence supports previous evidence (7, 20) that a multiplicity of substances are being measured. Although we have found the use of a darkroom advantageous, the products remaining after partial destruction by light appear to be stable (3). Modifications of the ethylenediamine technic, which do not require the use of a darkroom, appear to be satisfactory (5, 9, 21, 22). Whether the procedure is carried out in darkness or daylight, it is important to control this factor in order to obtain reproducible results. To avoid excessive destruction of samples during the reading, neutral density filters are used to reduce the intensity of the incident light.

In normal adults, plasma concentrations obtained with this modification of the ethylenediamine technic do not appear to differ significantly from those reported by others (4, 7, 9, 21–23). The results of our experiments, as well as those of other authors (4–7, 9), indicate that dopamine and dihydroxyphenylacetic acid are measured by this method. However, they would have to be present in rather large quantities in order to alter the results significantly. Dihydroxyphenylacetic acid and dihydroxymandelic acid have been isolated from bovine plasma by von Euler (9, 24) in amounts not sufficient to produce interference. Dopamine has not been isolated from peripheral human or bovine plasma in spite of repeated attempts (3, 7, 9, 24), but the possibility that this substance interferes with the determination of epinephrine is not completely excluded.

Although epinephrine levels in adrenalectomized dogs have been consistently negligible, studies of patients with adrenal insufficiency (Table 3) indicate that the amount of "apparent" epinephrine present in these patients is not significantly different from that observed in normal subjects. This could be due to the presence of dopamine or an extra-adrenal source of epinephrine in man. It is noteworthy that 8 of the patients had been previously subjected to bilateral total adrenalectomy. The levels in this group were 3.2 μg. per liter for norepinephrine and 0.47 μg. per liter for epinephrine. In addition to possible interference by chemically related substances, particularly in uremic patients, highly fluorescent substances such as vitamins, when present in very high concentration, may also interfere.

Some of the foregoing studies and others previously reported were performed in order to compare results obtained with this method to those reported by others using different chemical technics or bio-assay.

In contrast to the fall in epinephrine levels following insulin-induced hypoglycemia reported by Weil-Malherbe and Bone (25), we found a significant increase in normal subjects, psychiatric patients (13), and dogs (14).

Sympathetic activation has been shown to occur during electroconvul-

sive therapy. Studies carried out with this method indicate that unmodified electroconvulsive therapy is accompanied by a marked increase in plasma epinephrine and norepinephrine concentrations (11).

In the clinical studies, a significant elevation of plasma epinephrine levels without any change in norepinephrine levels was observed in hyperthyroid patients. Diller and Kilpatrick (26) also studied the urinary catechol excretion by bio-assay in patients with hyperthyroidism, and they too found an increase in the urinary excretion of epinephrine but no significant change in the excretion of norepinephrine.

The difference between the norepinephrine level in active laboratory workers and that in subjects at rest following a basal metabolism test is comparable to that described by Sundin (27). He found that raising subjects to an angle of 75° on a tilt-table doubled norepinephrine excretion but did not change epinephrine excretion. The difference in plasma norepinephrine was of similar magnitude to the difference in urinary excretion of amines in resting and in exercising subjects, as reported by Kärki (28). The elevation of plasma epinephrine and norepinephrine levels in severe exercise was similar to that noted in studies of the urinary catechol excretion of marathon runners (28) and in other types of vigorous exercise (28–30). In studying the epinephrine and norepinephrine content of plasma protein fractions (15), reasonable agreement between the determinations by bio-assay and fluorimetric technics was found.

The studies in patients with pheochromocytoma, as well as the reported plasma concentrations of epinephrine and norepinephrine in other patients with this entity (31, 16), indicate that a plasma method is suitable for the detection of these tumors. The advantage of obtaining specimens before and after administration of histamine to patients with paroxysmal hypertension has been reported (12, 16), and is also illustrated by our results in Patients 6, 7, 8 and 9 (Table 4). The difficulty encountered in making the diagnosis of pheochromocytoma by means of hormonal assay in patients with tumors secreting epinephrine intermittently has been underlined by the report of Litchfield and Peart (32) and is illustrated by our findings (Patient 7). Here exploration was delayed because of a urinary excretion rate consistently less than 100 μg. of total catechols per twenty-four hours, despite typical clinical episodes and a familial history of pheochromocytoma. The father of the patient had bilateral pheochromocytomas and a sister had a pheochromocytoma of the right adrenal.

In our experience, the technic has been entirely satisfactory for the determination of epinephrine and norepinephrine in adrenal venous plasma. However, the method as applied to peripheral plasma appears to find its greatest use in the diagnosis of pheochromocytoma, or in measuring by repeated sampling from the same patient, relatively large changes

occurring over a short period of time. The limitations of the method are the result of several factors: 1) Epinephrine and norepinephrine are extremely potent substances which are rapidly removed from the circulation. 2) In normal subjects, epinephrine is intermittently secreted and is present in minute amounts. 3) Infusion of this substance at rates which cause pronounced physiologic effects may result in plasma concentrations of only 1–3 μg. per liter of plasma. 4) Since plasma norepinephrine is derived primarily from sympathetic nerve endings in normal subjects, the plasma norepinephrine represents primarily the small amounts of humor which diffuse into the circulation at the receptor site before being metabolized. 5) Restrictions imposed by problems of chemical specificity as discussed previously. Although fluorometric technics are at present the most sensitive available, there are limitations to the degree of sensitivity attainable (8, 10).

Acknowledgment

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PLASMA EPINEPHRINE AND NOREPINEPHRINE LEVELS DURING INSULIN-INDUCED HYPOGLYCEMIA IN MAN*†

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ABSTRACT

Plasma epinephrine and norepinephrine concentrations were measured by the ethylenediamine technic before and during hypoglycemia in 11 patients undergoing insulin-coma therapy. In 6 studies carried out during the first week of treatment, plasma epinephrine levels increased from a mean value of 0.2 μg. per liter (control) to 1.4 μg. per liter following administration of insulin. In 9 studies carried out during the second and third weeks of therapy, the control values were significantly elevated (0.9 μg. per liter), although peak epinephrine concentrations reached after insulin were similar to those observed during the first week. In 9 studies performed in patients treated for more than three weeks, the control epinephrine concentration was 0.15 μg. per liter and no significant increase was observed during hypoglycemia. Norepinephrine levels remained unchanged in the 2½-hour period following administration of insulin in these subjects. However, a significant increase was noted in samples obtained between two and a half hours and four hours after insulin. The results of these studies indicate that the adrenal medullary response to hypoglycemia in man is associated with a rise in plasma epinephrine levels as measured by the ethylenediamine technic, and that this response is significantly altered by repeated insulin-induced hypoglycemia.

AN INCREASE in the circulating "sympathins" during hypoglycemia was demonstrated as early as 1924 (1–3). More recent studies, however, have not uniformly confirmed this observation. Heilbrunn and Leibert (4) in 1939 and Weil-Malherbe (5) in 1953 found a decrease in circulating epinephrine during insulin-coma therapy that appeared to parallel the changes in blood sugar. Holzbauer and Vogt (6) in 1954 meas-

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ured the epinephrine levels in peripheral plasma obtained from a human subject and from 2 dogs during insulin-induced hypoglycemia. Bio-assay of the adrenergic amines after their extraction from the plasma and chromatographic separation showed an increase in the plasma epinephrine content during hypoglycemia. Millar (7), in a study using a modification of the fluorometric technic described by Weil-Malherbe and Bone (8), reported similar findings. An increase in plasma epinephrine levels after administration of insulin has also been observed in dogs (9) and in a normal human subjects (10).

In contrast to these divergent results in peripheral plasma, experimental studies have consistently shown a depletion of epinephrine from the adrenal medulla during induced hypoglycemia (11–16). In dogs, measurements during insulin-induced hypoglycemia (9, 17) demonstrated a pronounced increase in the rate of secretion of epinephrine from the adrenal medulla. The urinary excretion of epinephrine is also increased in man under such circumstances (18–20).

Preliminary measurements in 4 patients, late in the course of insulin-coma therapy, indicated that plasma epinephrine levels were not increased during coma, despite very low blood sugar levels. Because of the discrepancy between these findings and those in the normal subject and experimental animals, we undertook a more extensive study of the plasma epinephrine, norepinephrine and blood sugar levels of patients in all stages of insulin therapy. The results are described in this report.

METHODS

The subjects were 11 schizophrenic patients selected for insulin-coma therapy on the basis of psychiatric indications. They were placed in bed on the insulin-therapy ward, without premedication, after an overnight fast. A single dose of crystalline insulin was administered intramuscularly, except as noted. The dosage was increased with successive treatments until the amount required to produce coma was established. Insulin was administered for five days, followed by two days of rest. The requirement for the induction of coma in these subjects ranged from 40 to 600 units of insulin. Treatments were terminated by the administration of glucose four to five hours after injection of insulin.

In each study a control sample of blood was obtained by venipuncture, immediately preceding the injection of insulin. Additional samples were then taken at intervals for three hours following the injection of insulin. The intervals between samples varied from twenty to sixty minutes because of technical difficulties encountered in patients who were unable to cooperate. However, an average of 5 specimens was obtained during each study.

Venous blood was placed in precooled centrifuge tubes containing heparin and then packed in ice. An aliquot was frozen for blood sugar determination by the Nelson modification of the Somogyi technic (21). The remainder of the sample was centrifuged, and the plasma was removed and frozen until analyzed for epinephrine and norepinephrine content by a modification of the ethylenediamine technic (10).

Twenty-four studies were made on the 11 patients. Six studies were performed during

the first week, 9 between the sixth and sixteenth day, and 9 between the sixteenth and fortieth day of treatment. It was possible to make sequential observations in 3 patients for five or more weeks. In one of the 3, insulin was withheld for one week after the third week of treatment. In another, insulin was withheld for a week after the fourth week of therapy.

RESULTS

Since administration of insulin by the intramuscular route at the initiation of therapy does not always produce a well-marked hypoglycemia, 4 patients were given insulin intravenously on the first day of treatment. In the first patient, injection of 10 units of insulin resulted in only a slight fall in the blood sugar level—to 55 mg. per 100 ml. This fall was associated with a slight increase (0.8 μg. per liter) in the plasma epinephrine level. In the remaining 3 patients, injection of 16, 20 and 20 units of insulin resulted in a drop in blood sugar levels to 27, 23 and 18 mg. per 100 ml. respectively. As shown in Figure 1, the mean plasma epinephrine concentration in these patients rose from a control level of 0.4 μg. per liter to a maximum of 2.1 μg. per liter in sixty minutes. During this period the mean blood sugar level fell from 82 to 22 mg. per 100 ml. When insulin was administered intramuscularly during the first week of treatment in amounts sufficient to cause a pronounced reduction in blood sugar levels, plasma epinephrine concentrations again increased. Figure 2 illustrates the response of plasma epinephrine levels to the intramuscular injection of 80 units of insulin into a patient on the fourth day of treatment. Although the peak response was reached only after one hundred and fifty minutes, the inverse relationship to the blood sugar level was again noted. Six studies were carried out during

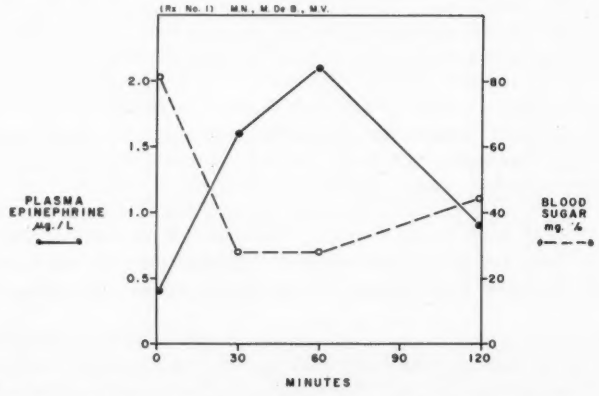

FIG. 1. The mean plasma epinephrine and blood sugar concentrations in 3 patients following the intravenous injection of 16, 20 and 20 units of crystalline insulin on the first day of treatment.

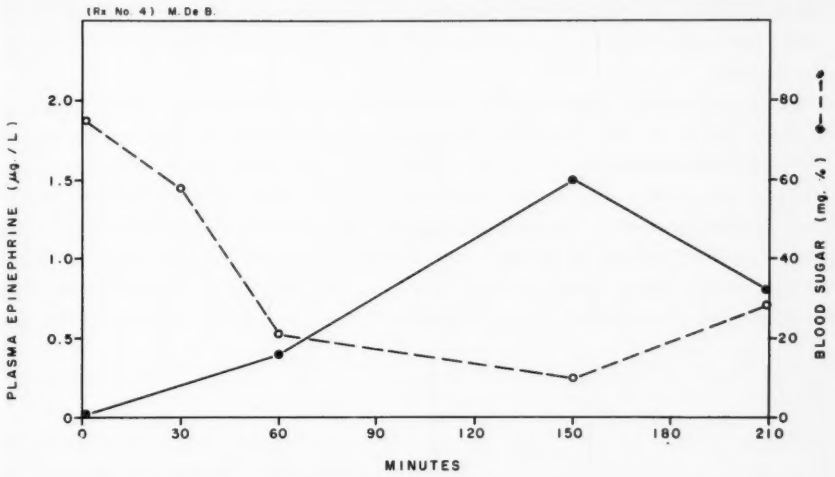

FIG. 2. Effects of 80 units of insulin administered intramuscularly on plasma epinephrine and blood sugar concentrations.

the first week of treatment, as shown in the first column of Figure 3. The mean control concentration of plasma epinephrine was 0.23 ± 0.32 μg. per liter, and the mean increase during the period of hypoglycemia was 1.2 ± 0.50 μg. per liter ($P < 0.001$).

In the 9 studies performed on the sixth to the sixteenth day of treatment, the mean of the maximal epinephrine concentrations during the hypoglycemic period (1.4 μg. per liter) was not significantly different from the maximal concentration observed during the first week of treatment: control levels, however, were uniformly and significantly elevated to 0.9 ± 0.1 μg. per liter ($P < 0.001$) (Fig. 3). An initial reduction in the plasma

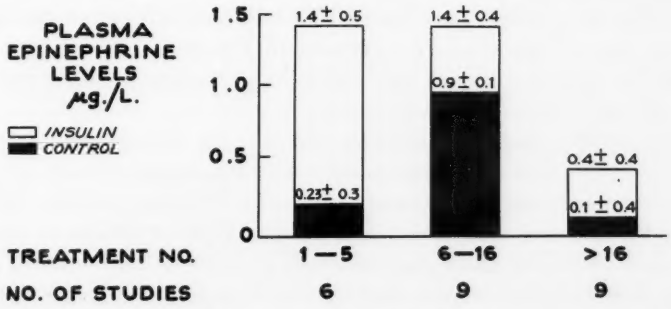

FIG. 3. Effect of repeated administration of insulin on the plasma epinephrine concentration in patients before and during hypoglycemia.

concentration of epinephrine following the injection of insulin, similar to that shown in Figure 4, occurred in 7 of the 9 studies carried out during this period.

Nine studies were carried out on 8 patients who were treated between 17 and 40 times. As shown in the third column of Figure 3, the mean control epinephrine concentration of 0.15 ± 0.37 μg. per liter was similar to that found during the first week of therapy. The mean of the highest levels during hypoglycemia (0.39 ± 0.42 μg. per liter) was not significantly different from that observed before the administration of insulin. Two of the 9 studies were carried out on the third day of treatment, following a one-week period without insulin. In each patient a response was noted during

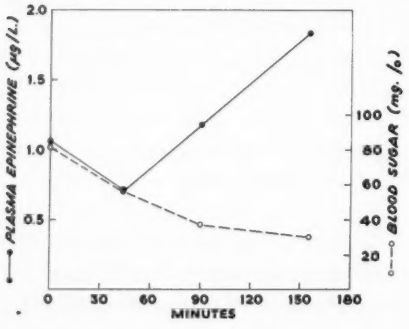

FIG. 4. Plasma epinephrine and blood sugar concentrations after the intramuscular injection of 80 units of insulin to a patient on the fourteenth day of coma therapy.

the week before, but not after, this period. Since studies were not carried out on the day following resumption of treatment, it is not known whether the patients responded on that day. In this group of observations, in which no significant increase of plasma epinephrine concentration occurred following insulin administration, the blood sugar concentration fell to a level of less than 15 mg. per 100 ml. in each instance. This alteration in response is best illustrated by the effects of the intravenous administration of 16 units of crystalline insulin to Patient M. DeB. on the first and seventeenth treatment days; these data are compared in Figure 5. Although the blood sugar levels were similar on both occasions, a marked difference in the plasma epinephrine levels was observed.

No relationship was noted between blood sugar and plasma norepinephrine levels in the two and a half hours following administration of insulin. In 112 determinations carried out during the foregoing studies, the mean norepinephrine level was 1.36 ± 0.72 μg. per liter, which does not differ significantly from the mean control levels in these patients and in normal subjects at rest, or the preconvulsive levels in patients undergoing electroshock therapy (22) (Table 1). In 12 plasma samples obtained two and a half to four hours after administration of insulin but before the adminis-

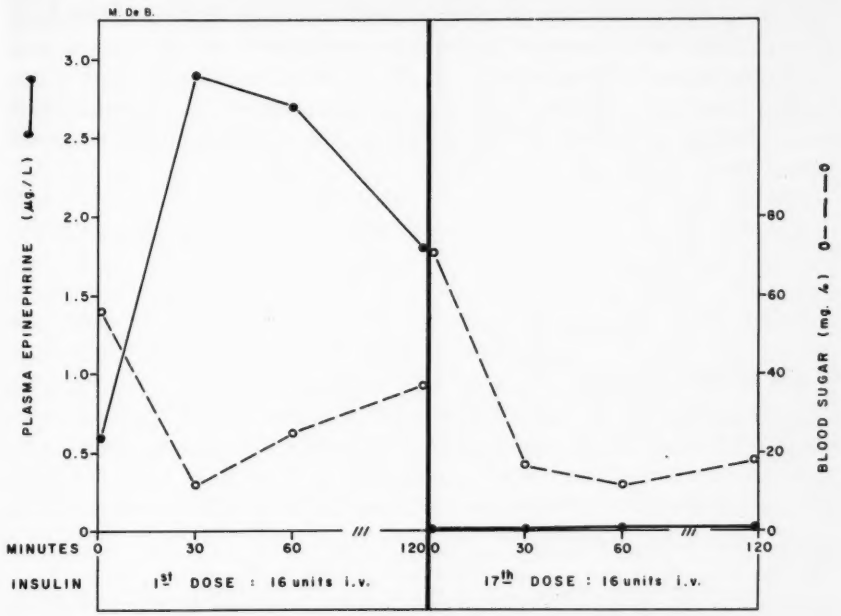

FIG. 5. A comparison of the effects of 16 units of insulin given intravenously to the same patient on the first and seventeenth days of therapy.

tration of glucose, the mean norepinephrine level was 4.3 ± 0.89 µg. per liter—a highly significant difference ($P < 0.001$).

DISCUSSION

The results of the foregoing studies are consistent with those showing an increased epinephrine output by the adrenal medulla during hypoglycemia, since plasma epinephrine is thought to be the product of medullary secretion in man. They also indicate that this increase in secretion of the

TABLE 1. COMPARISON OF PLASMA NOREPINEPRHINE CONCENTRATIONS IN NORMAL RESTING SUBJECTS, OTHER PSYCHIATRIC PATIENTS AND THE SUBJECTS OF THIS STUDY

Subjects	No. of determinations	Norepinephrine (µg./L. ±S.D.)
Normal subjects in basal state	12	1.55 ± 0.59
Patient undergoing electroconvulsive treatment (22); preconvulsive levels	85	1.45 ± 0.92
Subjects of present study:		
before injection of insulin	24	1.40 ± 0.72
0–2½ hours after insulin	112	1.36 ± 0.72
2½–4 hours after insulin	12	4.30 ± 0.89

medulla is reflected by an increase in peripheral venous plasma levels of epinephrine, when measured by the ethylenediamine technic—in contrast to the findings of Weil-Malherbe (5). The lack of response in some of our patients late in the course of insulin-coma therapy does not seem to account for the findings reported by Weil-Malherbe, since his study included several patients early in the course of treatment with insulin.

During the institution of insulin-coma therapy, the doses of insulin required to produce hypoglycemia with coma may be quite high. After the patient experiences his first coma, the dose of insulin required to produce subsequent comas may often be reduced. The hypothesis that this alteration is due to a change in the secretion of the adrenal medulla in response to hypoglycemia is not borne out by these results. In all cases, the lack of response was observed long after this alteration in the coma-producing effect of insulin had occurred. Moreover, in 2 patients who late in treatment repeatedly failed to become comatose in spite of marked hypoglycemia, there was no increase in venous plasma epinephrine levels. Since the samples obtained were of venous blood, a minor increase in the rate of secretion could have been obscured by the removal of epinephrine in peripheral tissues.

Although the lack of response of the adrenal medulla late in the course of treatment cannot be explained by the findings in our studies, it has been shown by several investigators (12–16) that following insulin administration the adrenal medulla becomes depleted of its amines for a prolonged period of time. The re-synthesis of epinephrine, in particular, is remarkably slow and may require as long as seven days, in contrast to the more rapid repletion of the gland with norepinephrine (12, 16). In 2 subjects in whom insulin was withheld for one week, studies were not carried out until the third day after treatment was resumed. At this time an increase in plasma epinephrine concentration was not observed.

It is of interest that lack of an increase in urinary epinephrine excretion during insulin-induced hypoglycemia has been reported in children with idiopathic hypoglycemia (23). The authors suggest that adrenal medullary failure is the cause of the syndrome. It would appear more likely, on the basis of the foregoing results and the rarity of spontaneous hypoglycemia in adrenalectomized patients maintained with adequate adrenocortical therapy, that the lack of response is the consequence of repeated episodes of hypoglycemia.

The increase in peripheral venous norepinephrine concentration in the samples obtained two and a half to four hours following administration of insulin is of special interest. During this period there was no change in the activity of these subjects and they remained hypoglycemic. An increase in ketone production has been observed three hours following insulin-induced

hypoglycemia (24), and available evidence indicates that an increase in activity of the sympathetic nervous system is associated with an increase in the mobilization of stored fat (25, 26). It is suggested that this increase in the level of plasma norepinephrine results in mobilization from adipose tissue of free fatty acids which are transported to the liver and there converted to ketones (27).

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SPECIFICITY OF THE TRIHYDROXYINDOLE METHOD FOR DETERMINATION OF URINARY CATECHOLAMINES*

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ABSTRACT

A wave length of 365 mμ for excitation of fluorescence in the trihydroxyindole method for urinary catecholamines was shown to be less specific than a wavelength of 405 mμ. Of 6000 urines studied, 256 were apparently abnormal when examined using 365 mμ, but 23 per cent of these were normal when examined with 405 mμ. Spectrophotofluorometric examination of activation and fluorescence spectra revealed occasional instances of falsely high results, even with 405 mμ. Further purification of the urine by treatment with ion exchange resin successfully removed the interfering material. Acid hydrolysis occasionally produced nonspecific interfering material. Normal values were obtained both for random samples and 24-hour urine collections. Moderate exercise did not cause any significant increase in catecholamine output.

THE trihydroxyindole fluorometric method for the determination of urinary catecholamines has, in recent years, gained widespread recognition as an important diagnostic test for pheochromocytoma. Over a period of eight years many thousands of such tests have been performed in the authors' laboratory and this experience has revealed the importance of one of the factors affecting the specificity of the test as usually performed. The purpose of this paper is to show by data accumulated in the performance of 6000 catecholamine assays that falsely elevated results, sometimes amounting to several times the maximal normal value, may be obtained through the use of improper excitation wavelengths. Data and discussion regarding various other aspects of the determination are also presented.

METHOD

The method of Sobel and Henry (1) was modified for this study as follows: Urine was collected with 0.4 Gm. of sodium metabisulfite and 0.2 Gm. of sodium fluoride per 100 ml. as preservative. A 5-ml. aliquot of urine (no hydrolysis) was centrifuged, and the

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clear urine adjusted to pH 8.5. It was then recentrifuged and passed through a $1 \times 3\frac{1}{2}$ cm. alumina column (Alcoa alumina, 60–200 mesh, washed free of fine particles with water and 0.2 *N* sodium acetate). The column was washed with 10 ml. of 0.2 *N* sodium acetate and 10 ml. of water, and eluted with 0.2 *N* acetic acid to a volume of 25 ml. Two-ml. aliquots of the eluate were taken for blank, sample, and internal standard. One ml. of water was added to the blank and to the sample tubes. One ml. of standard solution, containing 0.125 μg. of noradrenaline (free base), was added to the internal standard tube. The noradrenaline was obtained as the bitartrate from Winthrop Stearns. Na_2HPO_4 (0.5 *M*) was used to adjust the pH to 6.5 (about 0.8 ml. required) and oxidation was accomplished with 0.15 ml. of 0.25 per cent potassium ferricyanide for three minutes. This was followed, in the case of the sample and internal standard, by the addition of 1 ml. of 20 per cent sodium hydroxide-2 per cent ascorbic acid (9+1, freshly prepared) solution to convert the chromes to lutines. (Although preferred by some (2), manganese dioxide in our hands has not produced as reproducible readings and is too cumbersome a procedure when many samples are involved.) In the case of the blank, 0.9 ml. of 20 per cent sodium hydroxide was added; then, after waiting twenty minutes to allow for the destruction of the fluorescent lutines, 0.1 ml. of 2 per cent ascorbic acid was added. The ascorbic acid solution is unstable and must be prepared daily. All tubes were centrifuged and the fluorescence measurements were made within ten minutes of the addition of the last reagent. A Photovolt model 540 fluorometer was employed, with a high-pressure mercury lamp and primary filters to isolate the 365 mμ (Photovolt B Hg-1) or 405 mμ (Photovolt MM Hg-2 interference filter) lines in combination with the secondary filter (Photovolt B520). The secondary filter had a maximal transmission at 505 mμ.

All specimens calculated to contain catecholamines in a concentration of 18 μg. per 100 ml. or greater were reread on the Aminco-Bowman spectrophotofluorometer (ABSPF) with an Hanovia high-pressure xenon lamp as the light source, and using an activating wavelength of 405 mμ for scanning fluorescence spectra and a wavelength setting of 515 mμ on the fluorescent monochromator to scan the activation spectra. A 1P21 photomultiplier tube was used with both instruments. The fluorescence spectra were not corrected for response characteristics of the phototube.

EXPERIMENTAL DATA

Wavelength specificity

Typical blank and lutine fluorescence spectra for pure standards are illustrated in Figure 1. These curves, obtained on the ABSPF with 0.125 μg. of adrenaline (A) and noradrenaline (NA) in a total volume of 5 ml., indicate that noradrenolutine has an activation maximum at 400 mμ and a fluorescence maximum at 515 mμ and that adrenolutine has its activation maximum at 415 mμ and its fluorescence maximum at about 525 mμ. With proper adjustment of pH to 6.5 before ferricyanide oxidation, the two compounds gave approximately the same reading when excited at 405 mμ and the emitted radiation read at 515 mμ.

A typical set of curves for a urine containing no interfering substance is illustrated in Figure 2. The result, calculated with an activation wavelength of 365 mμ, was 16 μg. per 100 ml. With an activation wavelength of 405 mμ, the result was also 16 μg. per 100 ml.

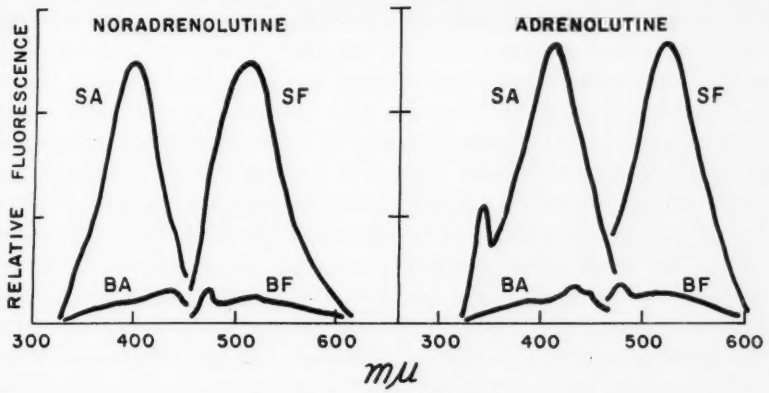

FIG. 1. Spectra of pure standards. B = blank; S = standard; A = activation spectrum, reading fluorescence at 515 mμ; F = fluorescence spectrum, activated at 405 mμ.

In a survey of some 6000 urine specimens analyzed by the routine procedure heretofore employed in this laboratory (*i.e.*, the use of the 365 mμ mercury line for excitation of lutine fluorescence), 256 samples were found to be abnormal (normal = up to 18 μg. per 100 ml.). Sixty of the "abnormal" values were reduced to the normal range when fluorescence was activated at 405 mμ. A pronounced deformity (Fig. 3) in the activation

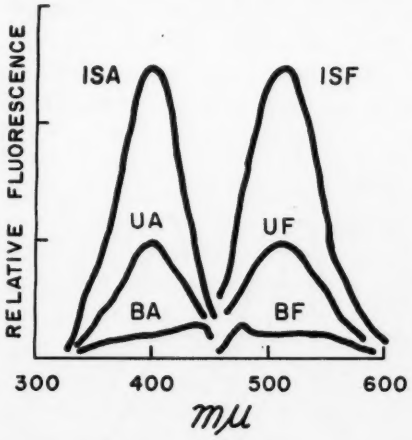

FIG. 2. Spectra obtained with a normal urine containing no interfering substance. B = blank; U = urine; IS = urine with internal standard; A = activation spectrum, fluorescence at 515 mμ; F = fluorescence spectrum, activated at 405 mμ.

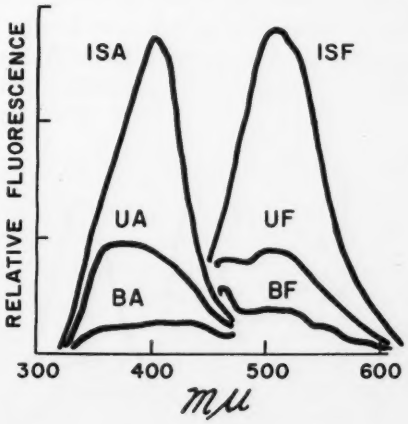

FIG. 3. Spectra obtained with a urine containing interfering substance activated at about 360 mμ. Abbreviations as for Figure 2.

spectrum of these urines at about 360 mμ demonstrated the source of these errors. In one case, a value of 80 μg. per 100 ml. was reduced to normal with the use of an activation wavelength of 405 mμ. The deformity in the activation curves of Figure 3 cannot be confused with the small peak at about 340 mμ in the activation spectrum for adrenolutine. In the other 196 cases, the fluorescence spectra showed no evidence of deformity, and the results calculated from the ABSPF at 405 mμ were essentially the same as the results obtained with the Photovolt using either the 365 mμ or the 405 mμ filter. Of 20 surgical or autopsy patients whose records were made available to us, 19 were found to have a pheochromocytoma. No tumor was found at autopsy in the twentieth patient, but the autopsy was limited to the adrenal region. The level of 3-methoxy-4-hydroxymandelic acid in this case was very high (19 mg. per liter). It cannot be concluded, therefore, that this was a false positive result. In the remaining 176 cases either no surgical exploration was performed or records were not made available. Thus, of 6000 urines studied, 256 yielded results that were apparently abnormal at 365 mμ, but 23 per cent of these proved to be normal when examined at 405 mμ.

Blanks and internal standards

The method calls for running a blank and an internal standard with each urine sample. The internal standard is used to correct for quenching, which may amount to 50 per cent. The avowed purpose of the blank is to correct for nonspecific fluorescence; but as already seen, the correction does not always succeed at 365 mμ. In 15 of the 6000 urines studied, the abnormalities observed were somewhat different from those just described. Three

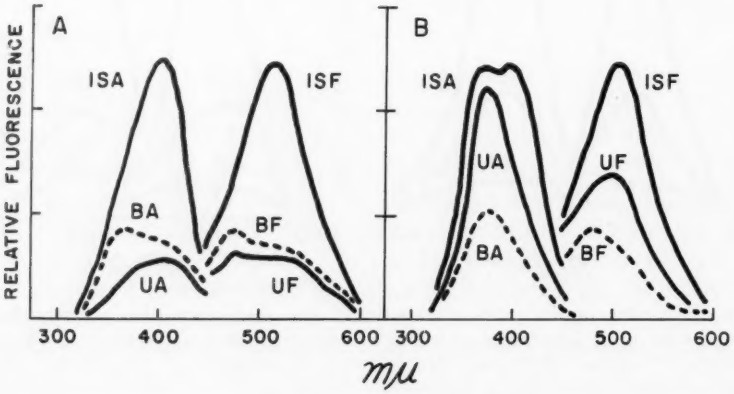

FIG. 4. Spectra obtained on urines giving high blank readings. Abbreviations as for Figure 2.

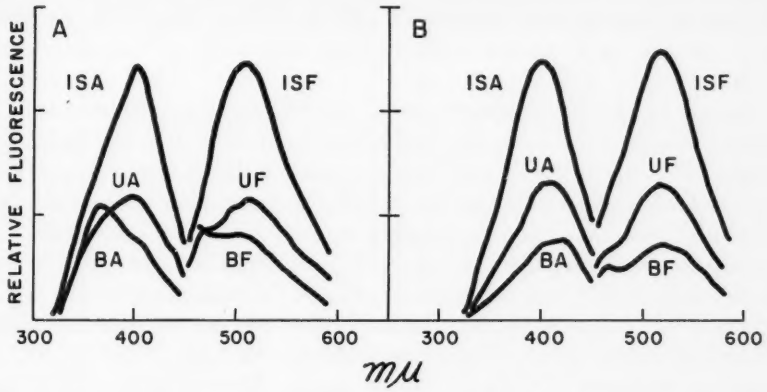

FIG. 5. Spectra of urine with marked quenching of internal standard, (A) before, and (B) after, resin purification. Abbreviations as for Figure 2. The curves in A and B represent different amounts of urine.

examples are illustrated in Figure 4A and B and in Figure 5A; in these instances, high blanks were obtained. Sample A in Figure 5A yielded a negative result at 365 mμ because the fluorescence of the blank activated at this wavelength was greater than that of the sample. At 405 mμ, however, a positive result was obtained. In the sample represented in Figure 4A the blank produced greater fluorescence than the sample at all wavelengths, yielding negative results at both 365 mμ and 405 mμ activation wavelengths. In the sample represented in Figure 4B, positive results were obtained with activation wavelengths of 365 mμ and 405 mμ, but the result at 405 mμ was not trustworthy because of the gross amount of interfering fluorescent material present with an activation peak at 370 mμ, obviously neither adrenaline nor noradrenaline. This is actually an exaggeration of the condition in Figure 3. Occasionally, in instances in which quenching of the internal standard was greater than 25 per cent at an activation wavelength of 405 mμ, the activation spectra for urine and the internal standard were distorted, raising doubt about the validity of the result. This occurred even when the blank values were very low.

Thus, it was previously established that results obtained with an activation wavelength of 365 mμ were frequently spuriously high and that use of 405 mμ markedly improved the specificity of the test. Now it has been shown that, in extreme cases, the results at 405 mμ may also be in error as a result of interferences. If fluorescence is measured routinely with a filter instrument, such as the Photovolt, the clue is a high blank and/or marked quenching of the internal standard (>25 per cent). In such a case, the complete activation and fluorescence spectra must be run and, if the curves

are such as illustrated in Figure 4A and B and in Figure 5A, accurate results cannot be obtained without removing most of the interfering material.

Removal of such interfering material was effected by resin treatment as follows: A column (1×4 cm.) of Amberlite CG50 resin, 100–200 mesh, was prepared from resin which had been treated for thirty minutes in a 1+1 mixture of 0.5 *M* Na_2HPO_4 and 0.5 *M* KH_2PO_4 containing about 3 per cent NaCl. The resin was then thoroughly washed with distilled water. The resulting pH of the resin was 6.5–7.0. The alumina eluate plus 0.1 volume of 4 per cent EDTA was adjusted to pH 6.5–7.0 with 1 ml. of the 0.5 *M* phosphate buffer and sodium hydroxide. After passing the alumina eluate through the column, the column was washed with 5 ml. of pH 6.5, 0.1 *M* phosphate buffer and then 10 ml. of water. The catecholamines were eluted with a volume of 0.5 *M* formic acid equal to the original volume of the alumina eluate. Aliquots of the formic acid eluate were adjusted with about 0.1 ml. of 20 per cent sodium hydroxide and 0.5 *M* Na_2HPO_4 to pH 6.5, and fluorescence was determined as previously described.

Figure 5 shows the findings on a urine containing interfering material—before and after resin purification. In order to yield results equivalent to those obtained from urine specimens subjected to alumina chromatography only (mean recovery 80 per cent), the results obtained after resin treatment are multiplied by 1.5.

Resin treatment has been used previously for purification of extracts (1, 3, 4).

Hydrolysis

Von Euler and coworkers (5, 6) have shown by hydrolysis at pH's near 0 that approximately 50 per cent of the catecholamines in urine are conjugated, and that the ratio of adrenaline to noradrenaline is the same for free and conjugated catecholamines. Significant hydrolysis was not observed at pH's greater than 2. These results were obtained by bio-assay.

Several normal urines were assayed for total catecholamines, both without hydrolysis and with hydrolysis for twenty minutes at 100° C. at different final acidities. The same urines with added noradrenaline (50 μg. per 100 ml.) were analyzed according to the same schedule. Typical results are shown in Table 1. No increase in catecholamine content was obtained at pH 2, but at pH 1 and lower, a significant increase in catecholamines was apparent. Hydrolysis, in a final concentration of HCl of 2.5 *N*, appeared to result in destruction of added noradrenaline. Hydrolysis in 1 *N* HCl resulted in the production of material yielding spurious fluorescence, as shown in the fluorescent curves in Figure 6. This spurious fluorescence may be corrected for by the urine blank, but it would seem wise to avoid a

TABLE 1. EFFECT OF HYDROLYSIS

Treatment	Catecholamines (μg./100 ml.)		Recovery (%)
	Urine alone	Urine + 50 μg. of noradrenaline/100 ml.	
No hydrolysis	12	51	78
Hydrolysis			
pH 2	11	50	78
pH 1	17	59	84
1 *N* HCl*	20	57	74
2.5 *N* HCl*	20	43	46

* Final HCl concentration.

procedure which adds to the nonspecific background fluorescence. The authors do not use hydrolysis in preparing urines for the routine determination of catecholamines.

Normal values

In Table 2 are listed the normal values obtained by the method presented. Urines were collected in bottles containing the preservative. Series 1 was composed of 6 males and 6 females. Series 2 comprised 16 adult males (22 analyses) and 17 adult females (23 analyses). Series 3 comprised 10 males and 11 females. Since the mean values for the males and females compared by the *t* test were not significantly different ($P > 0.1$), the values for males and females were pooled.

In series 1 the catecholamine concentration was significantly higher in the afternoon than in the morning, yet the excretion rate was the same, since the urine volume output was proportionately lower in the afternoon than in the morning. That the concentration in 24-hour urines was some-

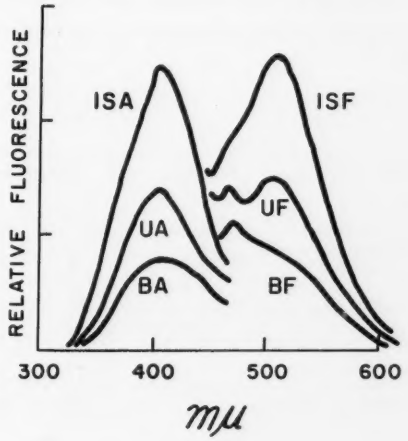

FIG. 6. Spectra after urine had been subjected to hydrolysis with 1 *N* HCl. Abbreviations as for Figure 2.

TABLE 2. NORMAL VALUES IN URINE

Series No.	Type of collection	Time of day	No. of subjects	No. of determinations	μg./100 ml.		μg./hr.		μg./24 hrs.	
					$\bar{X}$	95% limits	$\bar{X}$	95% limits	$\bar{X}$	95% limits
1	2-hr.	Morning	12	12	8.6*	2.4–14.8**	3.5†	1.8–6.8‡		
		Afternoon	—	12	11.9*	5.1–18.7**	3.2†	1.4–7.3‡		
2	Random	During day	33§	45§	8.6	0.5–16.7**				
3	24-hr.	—	21	21	7.4	4.0–13.9‡	2.8	1.3–4.3**	68	32–103**

* Difference between means for A.M. and P.M. significant by t test ($0.01 < P < 0.05$).
** Normal distribution.
† Difference between means for A.M. and P.M. not significant ($P > 0.1$).
‡ Lognormal distribution.
§ Series 2 includes random samples from 21 subjects plus the 24 samples from the 12 subjects comprising Series 1.

what less than in random day specimens could be predicted, since it has been shown that excretion at night is lower than in the day (7–9). The normal upper limit for random day samples was taken as 18 μg. per 100 ml.

The normal range for 24-hour excretion obtained in this study is in close agreement with that obtained by others using the trihydroxyindole method (10–13).

Effect of mild exercise on catecholamine levels in random samples

Von Euler and Hellner (10) showed that catecholamine excretion is increased following strenuous exercise, but not following mild or moderate exercise.

To determine whether ordinary ambulation might significantly affect the output of catecholamines in random urine samples collected after such exercise and, therefore, require consideration in interpretation of results, the following tests were performed: (*a*) Subjects collected a two-hour urine specimen, then drank two glasses of water and climbed stairs for ten minutes and again collected urine one to two hours later. (*b*) Subjects took a one-mile walk instead of the stair-climbing. As shown in Table 3, no clinically significant increase in catecholamine output was observed.

TABLE 3. EFFECT OF MILD EXERCISE

Type of exercise	Subject	Urine catecholamines			
		Before exercise		After exercise	
		μg./100 ml.	μg./hr.	μg./100 ml.	μg./hr.
Climbing stairs, 10 mins.	1	7	5	8	6
	2	2	3	6	6
	3	9	3	8	3
	4	14	4	14	4
	5	6	4	6	4
	6	6	4	3	6
	7	8	8	5	6
One-mile walk	8	3	3	3	7
	9	6	3	3	5

DISCUSSION

The primary purpose in the present study was to document the greater specificity for catecholamines obtained in the trihydroxyindole method by using a wavelength of 405 mμ rather than 365 mμ for excitation of fluorescence. That the wavelength of activation is important to specificity was deduced by Goldenberg and associates (14) whose technic was to calculate ratios for results obtained using the excitation wavelengths 365, 405 and 436 mμ as a check on the specificity of the test. Crout (15) reported distortion of fluorescence curves, using 365 mμ for activation. Thus, by obtaining complete activation and fluorescence spectra on samples containing elevated levels of catecholamines, it is possible in many instances to detect the presence of nonspecific fluorescence. In our experience, further purification on an ion exchange column will remove such interfering material.

Many workers have recommended the collection of 24-hour urine samples for catecholamine analysis. If there is intermittent secretion, however, the increased output during an attack would be diluted with the normal excretion during the remainder of the 24-hour period. The determination of catecholamine concentration in a random sample obtained following an attack should, in such an instance, be a more sensitive test. The results obtained on random samples in the present study appear to be as satisfactory as those on 24-hour collections for normal subjects. The use of a timed collection (such as a 24-hour specimen) is indicated, however, when the patient is excreting abnormal quantities of urine.

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EXCRETION OF 3-METHOXY-4-HYDROXY-MANDELIC ACID AND CATECHOL-AMINES IN PATIENTS WITH PHEOCHROMOCYTOMA

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ABSTRACT

Comparison of urinary catecholamine and 3-methoxy-4-hydroxymandelic acid (MHMA) excretions in established and suspected cases of pheochromocytoma demonstrated the confirmatory role of the MHMA test in diagnosis, and showed that in rare instances the excretion of MHMA may be elevated in association with an apparently normal output of catecholamines. A modification of published procedures for the estimation of urinary MHMA was employed. This method is suitable for multiple samples and is highly specific for MHMA. The adult normal range of excretion by this procedure was found to be 1.4 to 4.7 mg. per twenty-four hours.

WITH the demonstration of 3-methoxy-4-hydroxymandelic acid (MHMA) as a normal constituent of urine (1) and its identification as a metabolite of adrenaline and noradrenaline (2–7), several workers have reported elevated levels of this compound in cases of pheochromocytoma (2, 3, 8–10). The normal excretion of MHMA, as determined by paper chromatographic methods, is between 1 and 3 μg. per mg. of creatinine, corresponding to about 2 to 4 mg. per day (2, 3, 8–10). Other methods, presumably less specific, yield normal ranges that are two or three times as great (11, 12). The chemical specificity of the determination of catecholamines in the urine appears to be quite high when proper precautions are taken. The purpose of this investigation was to compare excretions of MHMA and catecholamines in proved and suspected cases of pheochromocytoma.

METHODS

Determination of urinary MHMA

A modification of the method of Armstrong *et al.* (3) was used employing hydrolysis for twenty minutes at pH 1.5 in a boiling water bath, extraction with ethyl acetate, and two consecutive one-dimensional chromatographic separations with simultaneous chromatography of authentic MHMA in the solvent systems (*a*) 2-propanol:ammonia

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(5 per cent w/v), 8:2 and (*b*) benzene:propionic acid:water, 100:70:5. Colors were developed on the papers by dipping quickly in a shallow layer of 0.1 per cent ethanolic 2,6-dichloroquinonechloromide, drying with warm air, dipping in a shallow layer of 0.2 *M* sodium borate, and once again drying with warm air. Spots were eluted with 0.1 *M* sodium borate and the solutions read at 590 mμ with suitable blanks and standards. Occasionally urines were encountered which contained a material having the same R_f as MHMA, but which imparted to the spots a hue different from that of MHMA alone when treated with the color reagent. This necessitated a third paper chromatographic separation with the system n-butyl alcohol:acetic acid:water, 8:2:2.

The two-dimensional paper chromatographic separation of many phenolic acids found in urine has been reported (1, 13). The proximity of several of the spots obtained with this technic and the similarity of colorimetric reactions for many of the compounds can cause some confusion for the analyst who must identify them by R_f measurements. For this reason, the authors resorted to the admittedly more time-consuming procedure of 2 one-dimensional chromatograms. Furthermore, the procedure allows for more convenient processing of multiple samples. A detailed description of the method is available from the authors upon request.

Recovery experiments and stability of samples. A series of 24 analyses yielded a mean recovery of 73 per cent (range, 60–88 per cent) for 5 μg. of MHMA added per ml. of urine. The clinical results reported here are not corrected for this mean 27 per cent loss. Half of these samples contained a bisulfite-fluoride mixture which is used as a preservative for catecholamines (14). No significant difference was observed in MHMA recovery or stability between the 2 groups. Samples were stable in the refrigerator and at room temperature for one week (Table 1).

TABLE 1. STABILITY AND RECOVERY OF URINARY MHMA

Urine No.	0 time		1 week at 4° C.		1 week at 25° C.	
	μg./ml.	Per cent recovery	μg./ml.	Per cent recovery	μg./ml.	Per cent recovery
1. Urine	1.1	—	1.5	—	1.5	—
Urine+preservative	1.0	—	1.4	—	1.4	—
Urine+5 μg. of MHMA/ml.	5.5	88	4.7	64	4.9	68
Urine+5 μg. of MHMA/ml. +preservative	4.8	76	5.4	80	5.3	78
2. Urine	2.0	—	2.1	—	2.2	—
Urine+preservative	1.6	—	2.2	—	2.0	—
Urine+5 μg. of MHMA/ml.	5.2	64	5.7	72	5.3	62
Urine+5 μg. of MHMA/ml. +preservative	4.6	60	6.2	80	6.0	80

All values are the means of duplicates.

Precision. In experiments involving 24 pairs of duplicate analyses in the range of 1–6 μg. of MHMA per ml. of urine, the standard deviation within a run was calculated to be ±0.17 μg. per ml., and the coefficient of variation ±5 per cent. The standard deviation between runs was ±0.48 μg. per ml. and the coefficient of variation was ±14 per cent.

Determination of urinary catecholamines

The method of Sobel and Henry (14) was employed, with several modifications introduced to improve the specificity and sensitivity of the method (15).

RESULTS

Normal values

Normal values were determined on 21 laboratory workers. Comparison of the data for the 10 males and 11 females showed no significant difference at the 5 per cent level by the *t* test. The results were, therefore, pooled. They were found by the normal equivalent deviate method (16) to conform to a lognormal distribution. The 95 per cent normal range after log transformation was 1.4 to 4.7 mg. per twenty-four hours. The coefficient of correlation between the 24-hour outputs of MHMA and catecholamines was 0.41, which was not significantly different from zero ($0.1 > P > 0.05$). Two of the male urines in this series contained material altering the hue of the MHMA spots, requiring the third paper system.

It is felt that calculation of MHMA excretion on the basis of creatinine output, as suggested by certain authors (3, 8, 12), is less desirable than calculation on a time basis. It has been established that the day-to-day variation of creatinine output for an individual may be as high as 25 per cent (17, 18).

MHMA and catecholamine excretion in suspected and proved cases of pheochromocytoma

Table 2 shows a comparison of MHMA and catecholamine excretions in 16 confirmed cases and 19 suspected cases of pheochromocytoma. Since 24-hour urine volumes were not available, the values for random samples are reported as mg. of MHMA and mg. of catecholamines per liter of urine. The coefficient of correlation between MHMA and catecholamine concentrations was 0.85, which is highly significant ($P < 0.001$).

A comparison of the results in Table 2 shows the marked increase above the normal MHMA excretion in confirmed cases of pheochromocytoma. Information regarding the unconfirmed cases has not been made available.

It was more common for the catecholamine levels to show the more striking increase in the abnormal urines. However, attention is called to sample No. 1d (Table 2), in which a normal catecholamine was accompanied by an exceptionally high MHMA level. Similar observations have been made by others (9, 19). Further attention is called to Case 1 in which 5 urines were analyzed over a period of one week. The marked variability in excretion of catecholamines and MHMA is well illustrated in this 10-year-old boy. No tumor could be found on operation. The boy died and at autopsy a pheochromocytoma was found under the diaphragm.

During the present study, this laboratory received urine specimens collected from a patient before and after operation for neuroblastoma. MHMA levels were 34.0 mg. and 2.8 mg. per twenty-four hours, respectively.

TABLE 2. MHMA AND CATECHOLAMINE LEVELS IN PATHOLOGIC URINES

	Case No.	Urinary (mg./L.)	
		MHMA	Catecholamines*
Proved cases of pheochromocytoma	1 a	51	2.36
	b	9	.14
	c	7	.17
	d	42	.17
	e	12	.11
	2 preop.	29	1.75
	postop.	3	.16
	3	84	1.95
	4	31	1.83
	5	34	.62
	6	20	1.56
	7	20	.67
	8	12	.49
	9	45	1.01
	10	12	.62
	11	42	.96
	12	16	1.80
	13	16	.38
	14 preop.	192	26.00†
	postop.	8	.11
	15	55	1.38
	16 a	27	1.15
	b	28	1.45
Suspected cases	17	27	3.96
	18	24	1.99
	19	73	5.82
	20	108	2.46
	21	29	1.55
	22	40	4.15
	23	6	.24
	24	13	.53
	25	29	2.50
	26 a	22	2.00
	b	91	3.86
	27	23	.70
	28	10	.36
	29	10	.31
	30 a	6	.27
	b	13	.78
	31	6	.27
	32	19	.20
	33	5	.41
	34	19	.57
	35	39	2.10

* Normal catecholamine level for random samples during daytime is 0–0.18 mg. per liter.
† Highest catecholamine level observed in more than 10,000 urine assays.

Comment

The determination of urinary MHMA appears to be useful in confirming an elevated urinary catecholamine output and in demonstrating tumor catecholamine secretion in instances in which the catecholamines are rapidly metabolized. It is also valuable as a primary test for pheochromocytoma. The fluorometric determination of catecholamines, however, remains far more expedient than the more involved paper chromatographic assay for the metabolite.

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URINARY EXCRETION OF NOREPINEPHRINE, EPINEPHRINE AND 3-METHOXY-4-HYDROXYMANDELIC ACID BY CHILDREN WITH NEUROBLASTOMA*†

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ABSTRACT

This study concerns the urinary excretion of norepinephrine (NE), epinephrine (E) and 3-methoxy-4-hydroxymandelic acid (VMA) in 5 infants and children with neuroblastomata and 1 child with a ganglioneuroblastoma. Prior to treatment by surgery, radiation or chemotherapy (alone, or in combination), 3 of the patients excreted increased amounts of both NE and VMA. One patient excreted slightly increased amounts of NE, but VMA output was very high. Another had a normal output of VMA, but a greatly elevated output of NE. In the remaining patient, both NE and VMA excretions were normal. The output of epinephrine was not increased in any of the cases. In general, clinical improvement following therapy was associated with a drop in the urinary excretion of NE and VMA. With progression of the disease, the excretion of these compounds again increased. These findings indicate that there may be biochemical differences among neuroblastomata, even though the tumors may be of similar histologic appearance. Delineation of these differences may be important in diagnosis, prognosis and treatment.

THE neuroblastoma is one of the most common malignant tumors of infancy and childhood. It generally results in a rapid downhill course and death in a few months, despite treatment by surgery, chemotherapy,

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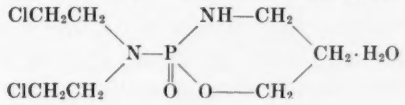
[1] Cytoxan® (Mead Johnson) is a potent cytotoxic agent related to the class of compounds known as "nitrogen mustards." It has the following chemical structure:



radiation, or a combination of these methods. However, in some cases the course is much different, and a complete cure is achieved even in patients with advanced disease. Koop *et al.* (1), Farber (2) and Wittenborg (3) reported apparent "spontaneous" cures. Fox *et al.* (4) described the maturation of a sympathicoblastoma into a ganglioneuroma in 2 patients who survived for twenty and forty-six years respectively. Gross *et al.* (5) recently reviewed 217 cases and discussed the many factors that were found to influence prognosis for a child with neuroblastoma. The reasons for the variations of response in patients with these neural tumors is not clearly understood. Conceivably, there are metabolic differences within the neoplastic cells which play an important role in differentiation, maturation and the response to therapy. Since norepinephrine has been shown to be the neurohormone of sympathetic nervous tissue, from which the neuroblastoma arises, a study of this catecholamine and its precursors and metabolites may provide information relating to the variations of response in cases of this tumor. The purpose of this paper is to report on the urinary excretion of norepinephrine (NE), epinephrine (E) and 3-methoxy-4-hydroxymandelic acid (VMA) in 6 patients who had either a ganglioneuroblastoma or a neuroblastoma.

MATERIALS AND METHODS

The 6 patients reported in this study comprise all of the cases with a diagnosis of neuroblastoma admitted to the pediatric wards of Syracuse Memorial Hospital from November 1959 to June 1960. Diagnosis was established in 5 cases by histologic identification of the tumor; in the remaining case there was a clinical picture consistent with neuroblastoma, and the tibial marrow aspirate revealed tumor cells in pseudo-rosette formation. Fourteen infants and children who were hospitalized for a variety of non-endocrine conditions served as controls. Urine specimens were collected during periods in their illnesses when catecholamine excretion was considered to be at physiologic levels, *e.g.*, prior to elective surgery, or late in convalescence. Studies were carried out on 24-hour urine specimens collected in bottles containing 8 to 15 ml. of 6*N* hydrochloric acid. Samples were kept on ice during the collection period and frozen immediately thereafter. Aspirin, bananas and vanilla-containing foods were omitted during the collection periods in order to avoid interference with urinary chromatographic analysis. Norepinephrine (NE) and epinephrine (E) were determined by a modification of the method of von Euler and Floding (6) using iodine as the oxidant. Fluorescence of solutions was measured in the Aminco Bowman spectrophotofluorometer. 3-Methoxy-4-hydroxymandelic acid (VMA) was estimated using the two-dimensional paper chromatography method of Armstrong *et al.* (7, 8).

RESULTS

Controls

Table 1 shows the urinary excretion of NE, E and VMA (μg. per twenty-four hours) in the control patients. The group is small, but this preliminary control study does demonstrate that age and weight must be considered in

assessing the normal range of excretion for infants and children. The 24-hour excretion of NE and E in our cases was similar to that reported by Kärki (9), Burn *et al.* (10) and Farquhar *et al.* (11). Von Studnitz (12) studied urinary VMA excretion in children aged 4–10 years using a method based on high-voltage electrophoretic separation. The excretion on the average was 2.3 mg. per twenty-four hours in these patients.

TABLE 1. URINARY NOREPINEPHRINE, EPINEPHRINE AND 3-METHOXY-4-HYDROXY-MANDELIC ACID EXCRETION IN 14 CONTROL PATIENTS

Patient		Urine volume (ml./24 hrs.)	Urinary excretion (μg./24 hrs.)		
Age—Sex	Body wt. (Kg.)		NE	E	VMA
4 mos. M	6	138	9.9	0.5	414
14 mos. M	9	405	10	0.8	405
20 mos. F	9.8	410	—	—	615
3 yrs. F	14.1	340	11	1.3	1360
3 yrs. M	12.7	250	9.2	1.0	1000
3½ yrs. M	12.4	750	15.7	9.1	1500
4 yrs. M	14	970	17.2	7.0	1940
4 yrs. F	14	325	12.4	1.9	1300
5½ yrs. M	16.5	1010	8.1	3.3	1010
7 yrs. F	23.3	590	—	—	1770
8 yrs. F	32.5	1095	—	—	2190
12 yrs. F	44.1	1045	22	6.0	—
13 yrs. M	50.9	1500	19	1.3	—
17 yrs. F	59	840	24.7	4.2	—

Children with Neuroblastoma—Case Studies

Case #1 (M.D.)

A 5½-year-old white male was admitted to Syracuse Memorial Hospital on April 7, 1960 with a history of swelling about the left eye and left jaw of two weeks' duration, as well as pain in his left thigh and a limp of two days' duration. Physical examination revealed a well nourished child weighing 17 Kg. He had a firm nonmovable mass (2×2 cm.) above the medial portion of the left eye, a firm nonmovable mass (4×5 cm.) in the left pre-auricular area, and a mass extending two fingerbreadths below the left costal margin. Blood pressure was 105/75 mm. Hg. An intravenous pyelogram demonstrated a suprarenal mass (5×6 cm.) depressing the left kidney and kinking the left ureter. A bone survey, including the skull, chest, pelvis and knees, showed no evidence of metastases. Examination of aspirated sternal marrow revealed tumor cells. Intravenous nitrogen mustard (7.2 mg.) and radiation to the facial masses were administered preoperatively. On April 14, 1960, Dr. Lawrence K. Pickett removed a large, soft mass weighing approximately 200 Gm., along with the spleen and left kidney to which it was adherent. There were no visible abdominal metastases. Tissue diagnosis was neuroblastoma (Fig. 1). The postoperative course was uneventful and the patient was discharged on April 23, 1960. Roentgen therapy was given to the left orbit, to the left parotid, and to the abdomen.

Progress of the disease was evident within six weeks. A small nodule appeared above the right eye and the child complained of pain in his legs. He was re-admitted to the hospital on June 8, 1960. Blood pressure was 84/60. In addition to the nodule above the

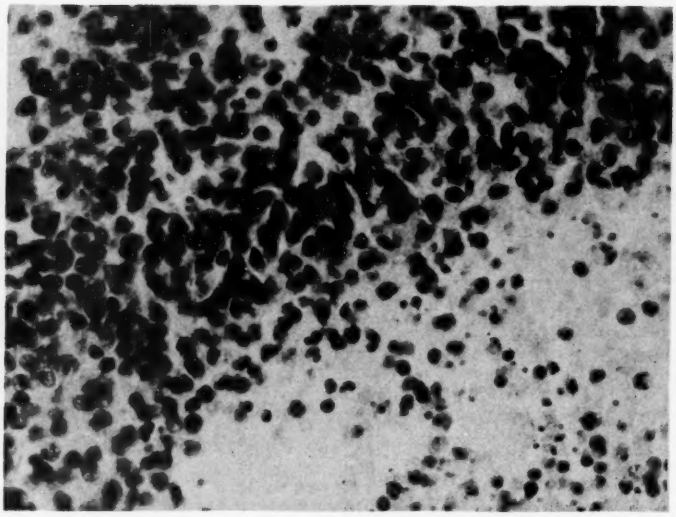


FIG. 1

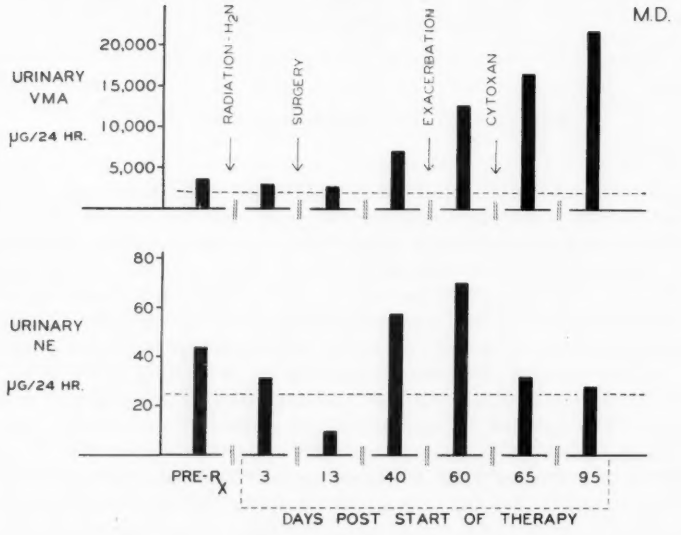


FIG. 2

FIG. 1. Section of neuroblastoma from Case 1 (reduced from ×500).
FIG. 2. Urinary excretion of norepinephrine and 3-methoxy-4-hydroxymandelic acid in Case 1. (The dotted line denotes normal values.)

right eye, many small nodes were present over the body. No abdominal masses were palpable. X-ray examination of the pelvis, hips and femurs revealed multiple areas of bone destruction. Intravenous administration of Cytoxan[1] (45 mg. daily) for seven days led to marked clinical improvement for about three weeks. Then progressive deterioration ensued. The child is doing very poorly at the time of this writing (August, 1960).

Comment. The urinary excretion of NE and VMA during the course of the disease is shown in Figure 2. There was an increased excretion of both compounds during the pretreatment period. After chemotherapy, radiation and surgery, the values returned to normal limits. Four to six weeks later, when there was clinical evidence of further metastases and progression of the disease, the excretion of NE and VMA rose to levels higher than during the pretreatment period. Cytoxan therapy was followed by a decrease in the urinary NE output, but VMA excretion continued to increase as invasion by the neuroblastoma proceeded. Epinephrine excretion remained within normal limits.

Case #2 (S.R.)

A 13-month-old white female was admitted to Syracuse Memorial Hospital on April 28, 1960, with a history of intermittent fever, abdominal distention, and an increase in number of stools from one to six per day for at least a month. Appetite and activity had been good until three days prior to admission. Physical examination revealed a thin child weighing 8.8 Kg. with a blood pressure of 145–180/110–130 mm. Hg. Small cervical and axillary nodes were present. The abdomen was markedly protuberant with bulging of the flanks. A firm, smooth mass extended from the right costal margin to the iliac crest. An intravenous pyelogram showed an extensive mass in the upper abdomen which displaced both renal shadows and ureters laterally and downward. A 4- to 5-mm. shadow resembling calcification was present in the right upper quadrant overlying the medial end of the eleventh rib. A bone survey for metastases demonstrated extensive destruction involving the frontal and parietal bones of the skull, both humeri, radii and ulnae, and the proximal metaphysis of the right tibia. There was a paratracheal mass on the right, and disease of the paraspinal soft tissues was evident bilaterally. Examination of a tibial marrow aspirate revealed tumor cells with morphologic characteristics similar to neuroblastoma. Intravenous nitrogen mustard (3.4 mg.) was given, and the child was discharged from the hospital on May 2, 1960. Subsequently, she improved somewhat for three or four weeks but remained hypertensive. Then a progressive downhill course ensued. The patient is doing very poorly at the time of this writing despite oral Cytoxan therapy.

Comment. Table 2 shows that urinary NE and VMA excretions were markedly elevated on each determination. The excretion of E was normal. Concomitantly, hypertension persisted and there was no significant remission of the disease.

Case #3 (L.S.)

A 13-month-old white female was admitted to Syracuse Memorial Hospital on December 11, 1959, with a history of chronic diarrhea of three months' duration re-

TABLE 2. URINARY EPINEPHRINE, NOREPINEPHRINE AND 3-METHOXY-4-HYDROXY-MANDELIC ACID IN CASES 2, 5 and 6 (NEUROBLASTOMA)

Patient	Date	Urinary excretion (μg./24 hrs.)		
		E	NE	VMA
(A) Case 2	Apr. 29, 1960	4.6	190	105,000
	Apr. 30, 1960	1.7	181.9	128,625
	May 1, 1960	0.06	169	127,500
	June 18, 1960	1.7	151.9	>100,000
(B) Case 5	Nov. 25, 1959	0.6	14.7	8,000
	Nov. 30, 1959	0.63	13.4	28,750
(C) Case 6	Dec. 31, 1959	0	3.1	—
	Jan. 2, 1960	—	—	500
	Jan. 5, 1960	2.4	18	570
	March 3, 1960	0.4	12.3	690

fractory to treatment with various medications and diets. For four to six weeks an intermittent rhinorrhea and flushing of the face had been present. Two days before admission a mass was felt in the left lower quadrant and surgical evaluation was advised. Physical examination revealed a healthy-appearing 9-Kg. child with a blood pressure of 110/40 mm. Hg. Pertinent findings included a malar flush, macular rash across the forehead and at the hairline, a greenish yellow nasal discharge and a firm, irregular mass (3×4 cm.) deep in the left lower quadrant. An intravenous pyelogram revealed no abnormality. Blood pH was 7.37. The serum bicarbonate level was 13 mEq., chloride 103 mEq., sodium 140 mEq. and potassium 3.7 mEq. per liter. A screening test showed no increase in urinary 5-hydroxyindoleacetic acid (13). A bone-marrow aspirate smear, chest roentgenogram, barium enema roentgenogram and stool cultures were all normal. On December 15, 1959 an exploratory laparotomy was performed by Dr. Lawrence K. Pickett. A firm, irregular 25-gram mass (approximately 7×3×2 cm.) was found at the bifurcation of the aorta, together with several enlarged mesenteric nodes. All visible tumor tissue was removed. The pathologic diagnosis was ganglioneuroblastoma (Fig. 3). No diarrhea, malar flush or facial rash was present in the postoperative period. The patient made an uneventful recovery and has been symptom-free since discharge on December 24, 1959. She received roentgen therapy to the abdomen postoperatively.

Comment. There was a marked increase in the urinary excretion of NE and a slight increase in the excretion of VMA preoperatively as shown in Figure 4. Following removal of the ganglioneuroblastoma, the values of both these compounds returned to normal. Serial determinations during the postoperative period showed no rise in excretion, although the values for NE and VMA were somewhat higher than in comparable controls. Epinephrine excretion was consistently normal. The diarrhea, facial flush and rash did not return during this period.

Case #4 (N.S.)

This 4-year-old white male was first admitted to Syracuse Memorial Hospital on May 21, 1959, because of swelling in his neck. Physical examination revealed a blood pressure of 118/60 mm. Hg, left-sided facial paralysis and cervical adenopathy. An in-

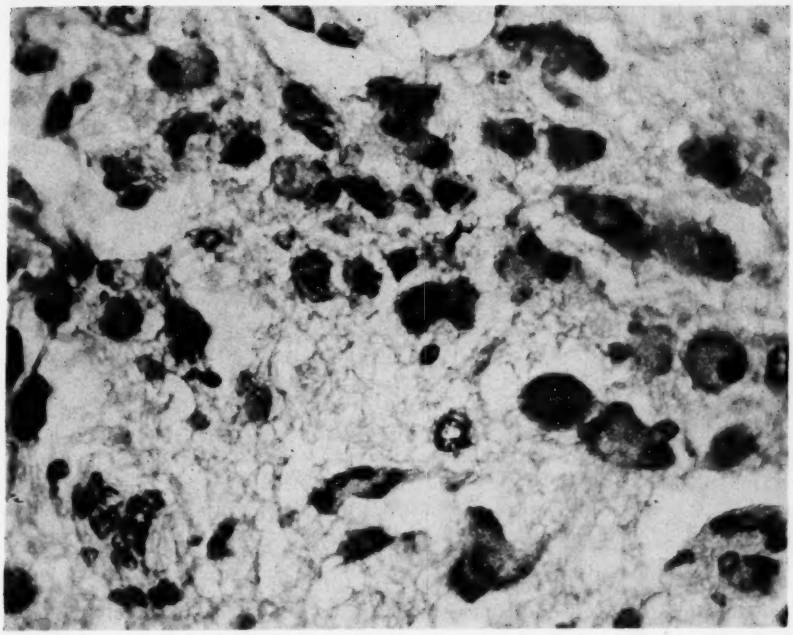


FIG. 3

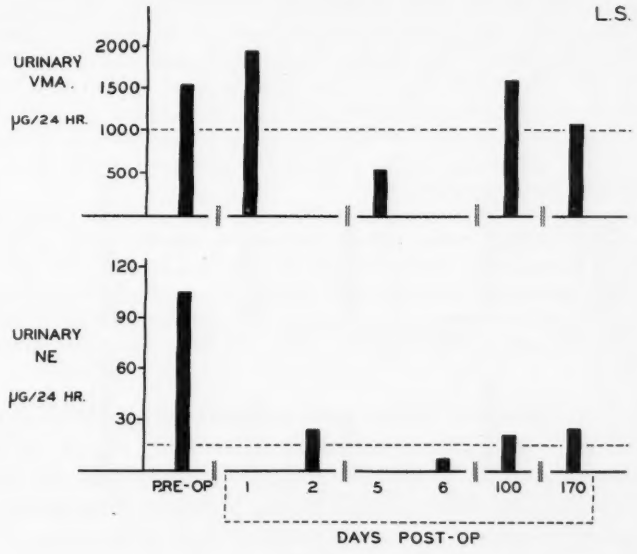


FIG. 4

FIG. 3. Section of ganglioneuroblastoma from Case 3 (×500).
FIG. 4. Urinary excretion of norepinephrine and 3-methoxy-4-hydroxymandelic acid in Case 3. (The dotted line denotes normal values.)

travenous pyelogram demonstrated an apparently calcified tumor lying adjacent to the upper pole of the left kidney. Mastoid films showed spotty destruction on the left. Left cervical node biopsy revealed a highly malignant tumor described as "most likely a neuroblastoma" (Fig. 5). Therapy consisted of 6.8 mg. of nitrogen mustard intravenously and radiocobalt therapy to the base of the skull.

The child did well until November 1959, when he was readmitted with a history of headaches, anorexia and dysphagia of several weeks' duration. Two weeks previously it had been noted that his left eye turned in and that there was a mass in the left side of the neck. On the day of admission the child lost consciousness for several minutes with no associated convulsive movements. Pertinent physical findings included: weight 16 Kg., blood pressure 160–180/100–120, left esotropia, and deviation of the tongue to the left. There was also a firm freely movable mass (3×3 cm.) in the left anterior triangle of the neck, and a firm hard mass indistinguishable from the liver extending from the right costal margin to the umbilicus and across to the left midclavicular line.

Skull and mastoid films showed no concrete evidence of bone destruction. Chest and spine roentgenograms revealed a bilateral paraspinal mass extending from the sixth dorsal vertebra down to at least the tenth dorsal vertebra, with no evidence of destruction of the vertebral bodies. Soon after admission to the hospital, the patient had intermittent episodes of flushing associated with complaints of being hot, during which time he completely undressed himself.

Therapy consisted of 6.4 mg. of intravenous nitrogen mustard and radioactive telecobalt therapy to the left head and neck. The patient improved remarkably. His blood pressure became normal, the flushing episodes disappeared, and the masses in the neck and abdomen decreased in size. He was discharged on December 12, 1959.

On December 30, 1959, he was readmitted because of anorexia, weight loss and severe headaches. Physical examination revealed an acutely and chronically ill patient with a rectal temperature of 102° F., pulse 140 per minute and respirations 46 per minute. Blood pressure was 160–170/80–110. Pertinent findings included paresis of the left sixth, seventh and twelfth cranial nerves, dullness over the lower half of the right lung, and a markedly protuberant abdomen with a fluid wave. There was a palpable mass extending 4–5 cm. below the right costal margin and 3–4 cm. below the left costal margin, and marked wasting of the extremities.

Thoracentesis of the right chest yielded 360 ml. of bloody fluid. The patient received radioactive cobalt therapy to the anterior epigastrium and the posterior epigastrium. Blood pressure decreased to 120–140/80–90. The abdominal distention and the mass decreased. Fluid re-accumulated in the right pleural cavity within a few days. The child was discharged somewhat improved on January 25, 1960. Soon his course became progressively downhill, and he died at home in February 1960. There was no postmortem examination.

Comment: Catecholamine studies were initiated during the second admission to the hospital. Figure 6 represents a summary of the findings. NE excretion was high prior to nitrogen mustard therapy, and decreased subsequently; it did not reach normal levels, however. Simultaneously, the blood pressure decreased to normal values. With progression of the disease, NE excretion increased and the hypertension returned. VMA excretion was not abnormal during the period of study, although there was a decrease in excretion following chemotherapy.

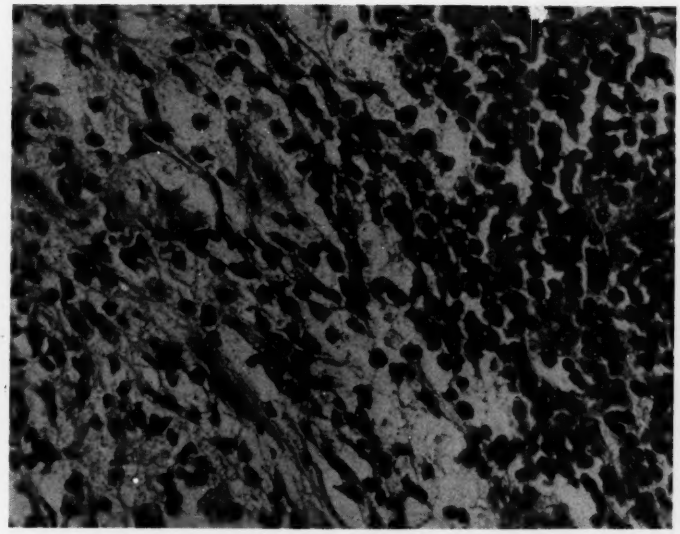


FIG. 5

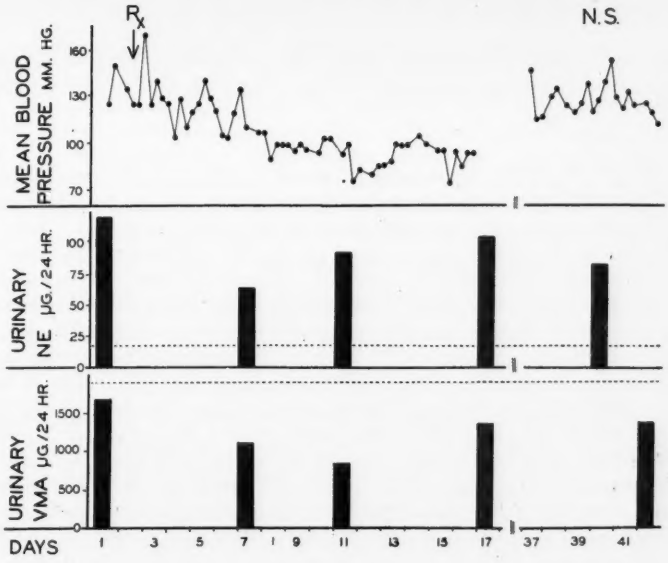


FIG. 6

FIG. 5. Section of neuroblastoma from Case 4 (reduced from ×495).
FIG. 6. Mean blood pressure and urinary excretion of norepinephrine and 3-methoxy-4-hydroxymandelic acid in Case 4. (The dotted line denotes normal values.)

Case #5 (R.C.)

A 7-week-old white male was admitted to Syracuse Memorial Hospital on November 23, 1959, with a history of paralysis of the lower extremities which had been noted shortly after birth. The parents described the baby as "pear shaped," with relaxed abdominal walls. Constipation had been a problem since birth. Pertinent physical findings included: weight 3.1 Kg., blood pressure 84/20 mm. Hg, widely bulging flanks and no tone to the abdominal musculature. There was one abdominal mass extending three fingerbreadths below the right costal margin, and another palpable 3 cm. below the left costal margin. The infant exhibited loss of motor and sensory function below the umbilicus, and there were cutaneous masses across the left costovertebral angle, behind the right ear and in the rectus sheath near the umbilicus.

X-ray examination revealed a large mass on both sides of the spine extending from the fifth or sixth dorsal vertebra to the second lumbar vertebra. No bone destruction was seen. There was moderate pleural effusion on the left. Biopsy of a nodule from the rectus sheath showed histologic evidence of neuroblastoma (Fig. 7).

The patient was treated with vitamin B_{12}, radioactive cobalt and supportive care. Severe respiratory distress and abdominal distention ensued. The patient deteriorated rapidly and died on December 8, 1959. Postmortem examination revealed neuroblastoma tissue apparently arising from the lumbar sympathetic chain and extending from below the bifurcation of the aorta to the diaphragm. There were metastases to the thymus, sternum, ribs, adrenals and liver. The liver metastases were confined to nodules seen on the external surface. The cut surface of the liver showed no metastatic lesions. The brain was not examined.

Comment: Table 2 shows that there was a slight increase in urinary NE and a pronounced increase in urinary VMA. The excretion of E was normal.

Case #6 (A. McG.)

This 7-month-old white female was admitted to Syracuse Memorial Hospital on December 27, 1959, because of a mass in the left abdomen noted two days before admission. Physical examination revealed a pale, chubby infant weighing 8.1 Kg., with a rectal temperature of 103.2° F. and a blood pressure of 134/84 mm. Hg. The left flank was visibly distended and palpation revealed a grapefruit-sized firm, round mass. An intravenous pyelogram demonstrated a large mass at the upper pole of the left kidney as well as rotation of the left calyceal system 90° in a clockwise fashion. An irregular calcification was observed at the level of the posterior interspace between the ninth and tenth vertebrae on the left. There was marked dilation of the right calyx. A retrograde pyelogram confirmed these findings. A chest roentgenogram was normal. On December 30, 1959, an exploratory laparotomy was performed by Dr. Lawrence K. Pickett. A large, soft, friable mass arising from the left suprarenal area and extending down to the pelvic brim and across the midline to the right renal pelvis and diaphragm was found. Only a portion of the mass superior to the left kidney was "scooped out." The pathologic diagnosis was neuroblastoma (Fig. 8). A tibial bone marrow aspiration biopsy revealed no tumor cells. The patient was treated with radioactive cobalt therapy to the abdomen. The postoperative course was complicated by pneumonia in the right upper and middle lobes and by anemia. The blood pressure range was 128–156/70–92. The child was discharged improved on January 3, 1960.

On February 8, 1960 she was readmitted with fever, proptosis and ecchymosis of the right eye. There were multiple firm masses over the parietal and occipital areas of the

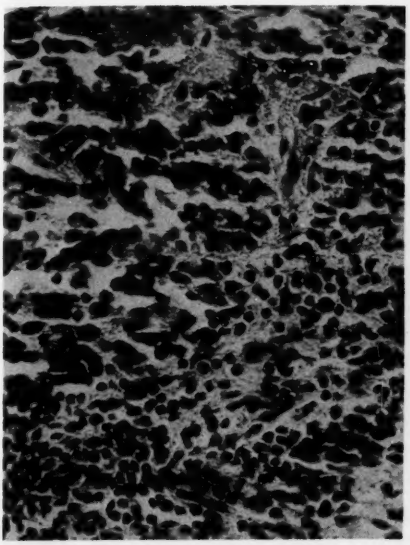


FIG. 7. Section of neuroblastoma from Case 5 (reduced from ×495).

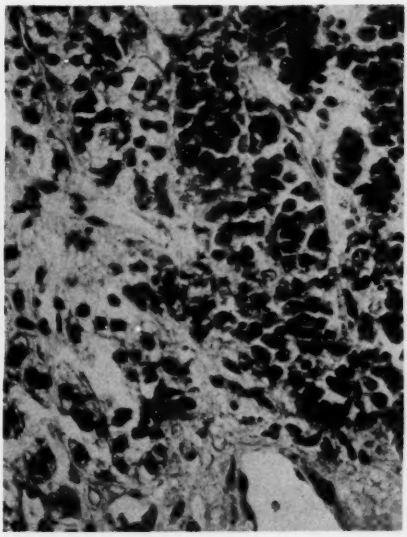


FIG. 8. Section of neuroblastoma from Case 6 (reduced from ×435).

head, with firm masses in the left neck. A mass extended two fingerbreadths below the right costal margin. Skull x-ray films revealed no bone destruction, but soft-tissue swelling was apparent. A right superior mediastinal mass was seen in the chest roentgenogram. There was little or no improvement following administration of 2.4 mg. of nitrogen mustard intravenously. Blood pressures remained in the range of 126–134/80–84. The patient's course was progressively downhill, and she died on March 5, 1960. Postmortem examination revealed far advanced abdominal carcinomatosis with extension of the retropleural nodes to the left subclavicular area and to the skull. The liver and brain were without evident lesions on microscopic examination.

Comment: Table 2 shows that the urinary excretion of E, NE and VMA was not significantly increased during any study period in this patient.

DISCUSSION

Increased urinary excretion of catecholamines or their metabolites, or both, has been reported recently in association with ganglioneuroma, ganglioneuroblastoma and neuroblastoma (14–23). These neoplasms are embryologically related, having a common origin from neural-crest ectoderm. Since norepinephrine is a principal neurohormone of the sympathetic nervous system, such findings are not surprising. Although the sympathetic nerves and ganglia readily synthesize norepinephrine, they are unable to synthesize epinephrine (24, 25). Thus urinary excretion of epinephrine in association with neural tumors would not be expected to be abnormally increased.

The principal pathway for biosynthesis of norepinephrine and epinephrine is shown in Figure 9.

The major route of metabolism of norepinephrine and epinephrine is shown in Figure 10.

Studies of catecholamine metabolism have demonstrated that urinary excretion of metanephrine and VMA generally exceeds the excretion of NE and E by 10-fold to 100-fold (26–31). Thus in Cases 1, 2 and 5 the presence of markedly elevated levels of urinary VMA as contrasted to NE and E indicates that normal metabolic pathways were in operation. How-

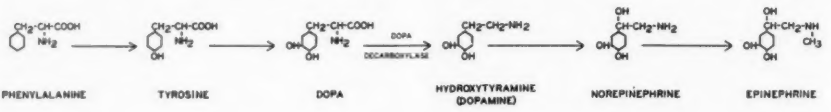


FIG. 9. Principal pathway for biosynthesis of norepinephrine and epinephrine.

ever, in Cases 3 and 4, although there was an elevated excretion of NE, the VMA output was not high. This suggests that there was inability to metabolize NE and E in the usual manner, leading to marked reduction in VMA formation. Such a departure from the usual metabolic sequence might be observed if there was alteration of the enzymes, catechol-o-methyl transferase, monoamine oxidase and aldehyde dehydrogenase (Fig. 10). Measurements of 3,4-dihydroxymandelic acid and metanephrine, under investigation in our laboratory, may aid in clarifying this point. The fact that large amounts of monoamine oxidase and catechol-o-methyl transferase have been found in liver (32, 33) may also be important. If a significant portion of the liver were replaced by neuroblastoma, it is possible that the metabolism of NE and E might be modified.

Urinary excretion of NE and VMA was within the normal range in Case 6 despite the fact that this patient had extensive involvement of tissues with neuroblastoma. There seem to be several alternate possibilities to account for such findings: 1) There was no increased production of catecholamine by the tumor because the neoplastic cells were too anaplastic to function in such a manner. 2) This tumor was unable to convert dopamine (or its precursors) to norepinephrine. Weil-Malherbe (34) and McMillan (35) suggested that some pheochromocytomas produce dopamine as the predominant catecholamine instead of NE. 3) The turnover rate for norepinephrine in the tumor was so slow that the urinary excretion of catecholamine was not increased. Sjoerdsma *et al.* (29) indicated that the turnover rate for NE in some pheochromocytomas may be more rapid than in others. Data are not yet adequate for drawing conclusions.

The hypertension in Cases 2 and 4 undoubtedly was due to elevated

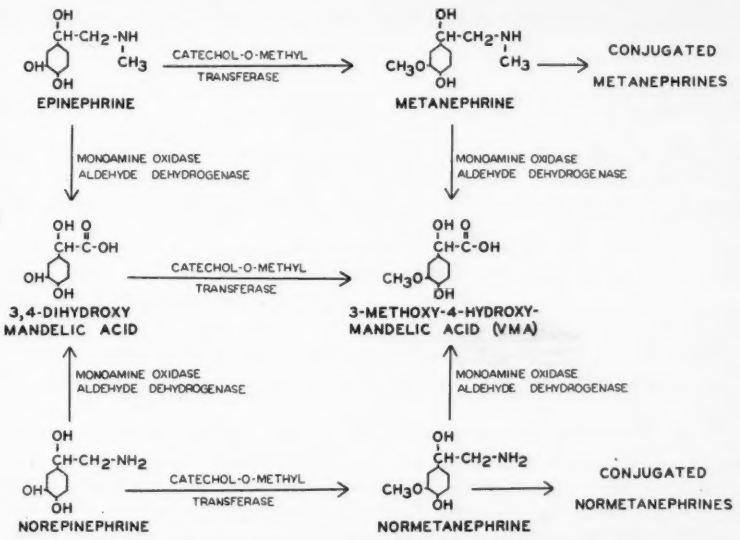


FIG. 10. Metabolism of norepinephrine and epinephrine.

production of NE in association with neuroblastoma. In Case 4, therapy resulted in dramatic relief of headache, a drop in blood pressure, cessation of paroxysmal attacks and reduction of urinary NE excretion. Headaches and hypertension returned with progression of the disease and again there was an increase in NE excretion. The clinical picture was not unlike that observed in cases of pheochromocytoma with excessive excretion of NE.

The presence of diarrhea in association with a ganglioneuroma or ganglioneuroblastoma (see Case 3) has been reported several times recently (17–20, 23, 36–38). Since the diarrhea ceased in the cases in which complete removal of the tumor was accomplished, there seems to be a cause-and-effect relationship between tumor and diarrhea. Our studies to date offer no biochemical explanation for this observation.

These preliminary studies of catecholamine metabolism in association with neural tumors extend previous knowledge because they indicate that there may be biochemical differences among neuroblastomas in which the histologic appearance does not permit further classification. These differences may be related to deviations from the principal pathways of the synthesis and metabolism of norepinephrine. Thus a biochemical classification of these tumors may have prognostic implications far more accurate than those based on histologic diagnosis. Likewise, delineation of metabolic differences may eventually permit the introduction of chemotherapeutic agents that may alter enzymatic pathways and be effective in treatment. Determinations of urinary NE, E and VMA also provide laboratory

criteria which are helpful in the diagnosis of neural tumors, and beneficial in following the patient during the course of the disease.

Acknowledgment

The authors wish to thank Dr. Lawrence K. Pickett, Dr. Nicholas M. Stahl, Dr. William J. Waters and Dr. Robert Shapiro for their assistance in obtaining specimens for this study. The technical help of Mrs. A. T. Lipfert and Mrs. J. Hey is appreciated.

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EFFECT OF THYROIDECTOMY ON PLASMA BUTANOL-EXTRACTABLE AND PROTEIN-BOUND RADIOIODINE LEVELS AND ON THE TURNOVER OF RADIOTHYROXINE

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ABSTRACT

In 5 of 7 euthyroid patients given radioiodine seventy-two hours before subtotal thyroidectomy there was approximately a two-fold increase in the plasma PBI^{131} level forty-eight hours after surgery. There was no change, however, in the level of BEI^{131}. In patients with Graves' disease prepared for operation with antithyroid drugs and given radiothyroxine, there was a sudden diminution in the rate of disappearance of radioactivity from the blood about six days after unilateral or bilateral subtotal thyroid lobectomy. The hypothesis is presented that manipulation of the thyroid at operation may sometimes release a significant amount of thyroglobulin which later undergoes proteolysis to release iodinated compounds including thyroxine. This proteolysis may occur close to, or at the site of the deiodination of thyroxine in the tissues, releasing a large local concentration of stable thyroxine which successfully competes with the labeled thyroxine and reduces its rate of deiodination and disappearance from the blood.

IT HAS been suggested that manipulation of the thyroid during thyroidectomy may release significant amounts of thyroid hormone and even cause postoperative "storm" (1–4). In one study, no change in the level of plasma protein-bound radioiodine (PBI^{131}) was noted in half an hour to two hours postoperatively in patients with nontoxic goiters (5). Others have reported an increase in the concentration of PBI in nontoxic (6, 7) and toxic (7) patients within twenty-four hours after surgery.

The present study was made to clarify the changes in the levels of PBI^{131} and butanol-extractable radioiodine (BEI^{131}), and the rate of degradation of radioactive thyroxine following thyroidectomy.

METHOD

Seven euthyroid patients with diffuse or adenomatous goiter were given carrier-free radioiodine (300 microcuries) orally seventy-two hours before unilateral or bilateral sub-

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total thyroidectomy. Blood was drawn one hour before operation, and forty-eight hours after operation. PBI^{131} concentration was determined by trichloracetic-acid precipitation of plasma (1). BEI^{131} concentration was determined by extracting acidified plasma three times with butanol, washing three times with Blau's solution (4*N* NaOH with 5 per cent Na_2CO_3), and evaporating (8). The radioactivity was measured in a well-type scintillation counter and expressed as a percentage of the dose.

The fractional rate of turnover of radiothyroxine was determined in the following groups: 1 euthyroid patient; 3 who were euthyroid during treatment for hyperthyroidism with mercaptoimidazole, methylthiouracil or x-ray therapy; 1 with untreated Graves' disease; 5 cases of Graves' disease treated with mercaptoimidazole, methylthiouracil and Lugol's solution who were clinically improved but still not euthyroid; and 3 with surgically treated hyperthyroidism (2 seemed euthyroid although in Case 12 exophthalmos had developed since the operation, and 1 was becoming thyrotoxic again ten years after surgery). The drugs were stopped at the time of operation.

Each patient was given 30–50 μc. of radio-*l*-thyroxine (3–5 μg.) intravenously. Daily blood samples were obtained and 24-hour urine collections made. The total radioactivity of the serum was measured in a well-type scintillation counter and expressed as a percentage of the dose of radio-*l*-thyroxine. *K* (the fractional rate of turnover of radiothyroxine) was determined by the method of Ingbar and Freinkel (9). In 2 additional patients, mercaptoimidazole was started thirty days before operation and was stopped just before operation, and Lugol's solution was started ten to fifteen days before operation and continued throughout the study. In 2 other patients the mercaptoimidazole and Lugol's solution were both continued throughout the study. Radiothyroxine was injected two hours before operation. Serum radioactivity was measured for the succeeding ten days in these last 4 patients.

RESULTS

Recoveries of known amounts of added radiothyroxine, radiodiiodotyrosine and radioiodine by the PBI and BEI procedures are recorded in Table 1. More than 80 per cent of the added radiothyroxine was recovered.

In 5 of the 7 euthyroid patients, the concentration of PBI^{131} increased between 1.4 and 4.7 times (average, 1.8 for all 7 patients) forty-eight hours after surgery compared to the level one hour before surgery (Table 2). In contrast, the BEI^{131} levels either remained virtually unchanged or were slightly lower (average ratio of postoperative to preoperative values, 0.87). In Patient S.M., who was clinically euthyroid, both PBI^{131} and BEI^{131} levels were elevated. There was no correlation between the type of surgery

TABLE 1. RECOVERY OF RADIOTHYROXINE, DIIODOTYROSINE, AND IODIDE BY THE PBI AND BEI PROCEDURES

Radioactive substance	Per cent of added radioactivity in protein-bound iodine	Per cent of added radioactivity in butanol-iodine
Thyroxine	84.4* ±1.05†	83.2 ±4.39
Diiodotyrosine	5.6 ±0.47	0.8 ±0.20
Iodide	0.1 ±0.03	0.84 ±0.12

* Mean value of 20 samples in each case.
† Standard deviation.

TABLE 2. PBI^{131} AND BEI^{131} LEVELS IN PERIPHERAL VENOUS PLASMA BEFORE, AND FORTY-EIGHT HOURS AFTER THYROID SURGERY IN EUTHYROID PATIENTS

Patient		Plasma PBI^{131} (% dose/L.)			Plasma BEI^{131} (% dose/L.)			Diagnosis	Operation
No. & initials	Age & sex	BO*	AO	A/B	BO	AO	A/B		
1) K.K.	28 F	0.049	0.088	1.8	0.059	0.042	0.71	Adenoma of thyroid	U**
2) K.H.	28 F	0.128	0.185	1.45	0.042	0.043	1.02	Nontoxic diffuse goiter	B
3) A.F.	46 F	0.038	0.071	1.92	0.031	0.036	0.97	Adenoma of thyroid	U
4) M.T.	36 F	0.032	0.024	0.75	0.034	0.024	0.71	Adenoma of thyroid	U
5) O.K.	17 F	0.037	0.175	4.73	0.033	0.028	0.85	Nontoxic diffuse goiter	B
6) S.M.	17 F	1.141	0.820	0.58	0.854	0.582	0.68	Nontoxic diffuse goiter	B
7) S.O.	40 F	0.101	0.144	1.42	0.080	0.088	1.10	Adenoma of thyroid	U

* BO = one hour before operation; AO = forty-eight hours after operation; A/B = the ratio between AO and BO.
** U = unilateral subtotal lobectomy; B = bilateral subtotal lobectomy.

and the change in PBI^{131} concentration. In 2 of the 7 patients the PBI^{131} level dropped.

The fractional rates of turnover of radiothyroxine (K) are recorded in, Table 3. In 1 normal euthyroid subject, 3 euthyroid subjects being treated for hyperthyroidism, and 1 euthyroid subject who had undergone subtotal thyroidectomy thirteen years before, the range of K values was 9.9–12.6 per cent per day (average, 10.9 per cent). In 1 patient with untreated Graves' disease, 5 with inadequately treated Graves' disease, 1 with a recurrence following surgery and 1 with surgically treated Graves' disease

TABLE 3. FRACTIONAL RATE OF TURNOVER OF THYROXINE IN VARIOUS STATES OF THYROID FUNCTION

Patient		Diagnosis	Therapy*	PMR		K (%/day)
No. & initials	Age & sex			BT**	BI	
1) U.S.	30 F	Euthyroid		2.0		10.25
2) W.H.	32 F	Treated hyperthyroidism; euthyroid	M, 0.36 Gm. for 18 days	14.0	10.0	9.9
3) K.S.	35 F	Treated hyperthyroidism; euthyroid	M, 0.45 Gm. for 30 days; T, 3.0 Gm. for 30 days; R, 120 r ten times	23.0	3.0	10.65
4) K.T.	22 F	Treated hyperthyroidism; euthyroid	M, 0.3 Gm. for 10 days; T, 1.0 Gm. for 10 days; R, 120 r four times	20.0	16.5	12.6
5) S.E.	24 F	Graves disease		28.0	28.0	16.12
6) W.H.	46 F	Treated Graves' disease; still toxic		37.0	25.5	17.3
7) S.T.	49 M	Treated Graves' disease; still toxic	M, 0.28–0.9 Gm. for 30 days;	39.0	34.0	18.3
8) F.F.	34 F	Treated Graves' disease; still toxic	T, 2.8–3.0 Gm. for 30 days;	39.0	28.3	18.3
9) M.H.	52 M	Treated Graves' disease; still toxic	Lugol's solution, 120 drops for 16 days	46.0	35.0	19.8
10) K.T.	39 F	Treated Graves' disease; still toxic		43.0	28.5	20.4
11) I.Y.	34 F	Operated on for Graves' disease; recurrence	Subtotal thyroidectomy 10 yrs. before; M, 0.3 Gm. for 10 days; R, 120 r four times	34.0	16.5	18.48
12) G.H.	57 M	Operated on for Graves' disease	Subtotal thyroidectomy 3 mos. before; thyroid powder 7.0 Gm. over 70 days	24.5	7.0	13.86
13) N.Z.	65 M	Operated on for Graves' disease; euthyroid	Subtotal thyroidectomy 13 yrs. before		7.0	11.0

* M = mercaptoimidazole; T = methylthiouracil; R = roentgen therapy.
** BT = before therapy; BI = before intravenous injections of radiothyroxine; K = fractional rate of turnover of thyroxine (proportion/day = $0.693/t_{\frac{1}{2}}$ (days)).

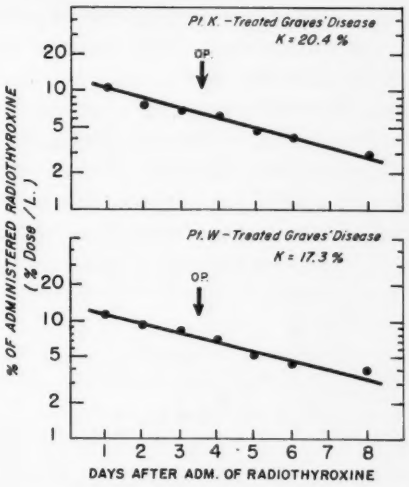

FIG. 1. The rate of disappearance of radiothyroxine (30–50 μc. intravenously) from the blood of patients with Graves' disease treated with antithyroid compounds and iodide. Operation had no immediate effect.

receiving 1½ grains of thyroid daily (for severe exophthalmos), the range of K values was 13.86–20.4 per cent per day (average, 17.8 per cent).

The patients with Graves' disease being treated with drugs in preparation for surgery were operated upon three to four days after injection of radiothyroxine. There was no immediate change in the rate of disappearance of radiothyroxine from the blood or in the rate of appearance of radioactivity in the urine (Figs. 1 and 2). The drugs had been stopped at the time of operation. In patients followed longer than four days after surgery, however, the rate of disappearance of radiothyroxine diminished at about day 5 or 6. Figure 3 shows the slopes of blood radioactivity (plotted as a percentage of the original dose of radiothyroxine) in 2 patients in whom Lugol's solution was continued throughout the study but mercap-

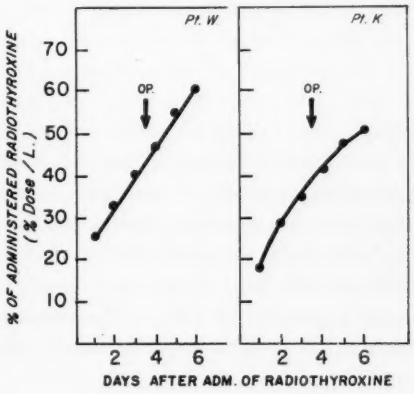

FIG. 2. Urinary radioactivity in the patients represented in Figure 1. There was no immediate change in excretion of radioactivity derived from radiothyroxine in these medically prepared patients with Graves' disease following thyroidectomy.

toimidazole was stopped just before operation. Figure 4 shows the slopes of blood radioactivity in 2 patients in whom both drugs were continued throughout the study. In the 4 patients represented in Figures 3 and 4, radiothyroxine was given two hours before operation, whereas in the earlier patients (Fig. 1) thyroxine was given three to four days before operation. In no patient did the thyroidal uptake of radioactivity following the injection of radiothyroxine exceed 5 per cent, so that extrathyroidal recycling of radioiodine was not significant.

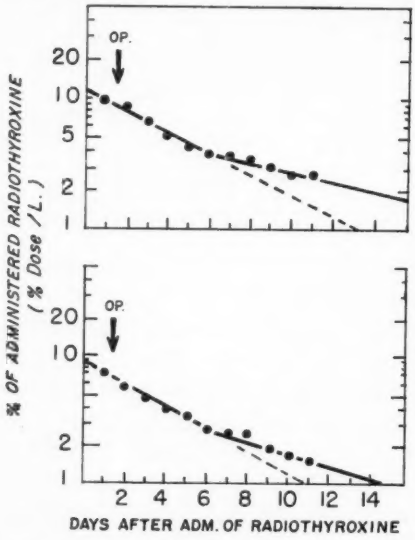

FIG. 3. The rate of disappearance of radiothyroxine from the blood of 2 medically prepared patients with Graves' disease. The rate decreased on the sixth day after thyroidectomy. Lugol's solution was continued throughout but mercaptoimidazole was stopped at the time of operation.

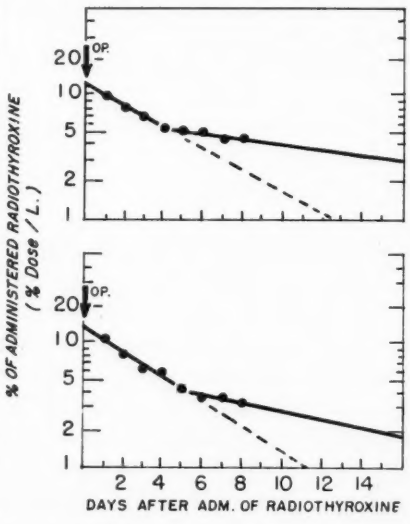

FIG. 4. Effect of continuing both Lugol's solution and mercaptoimidazole throughout the study in 2 patients of the type represented in Figure 3.

DISCUSSION

The increase in the concentration of PBI^{131} forty-eight hours after operation might have occurred in the absence of surgery, as PBI^{131} often continues to increase in the blood of euthyroid patients for more than five days after administration of radioiodine (10). It seems unlikely, however, that in patients who had undergone bilateral subtotal lobectomy, enough tissue would be left to supply an increasing amount of PBI^{131}. Conclusive evidence against the hypothesis that residual thyroid tissue was supplying

the PBI^{131}, was the lack of any increase in the concentration of BEI^{131}. Were the remaining thyroid tissue activated to secrete increasing amounts of PBI^{131}, the plasma concentration of BEI^{131} would also have increased.

The present evidence indicates that operative manipulation increased the level of PBI^{131} but not of BEI^{131}. This suggests that the increase in PBI^{131} was largely in the form of thyroglobulin or even more complex portions of thyroidal colloid. One would expect this increase to be apparent soon after surgery and therefore the results of Hydovitz (5) are puzzling—unless the operative technique was much rougher in the present study. It is of interest that in 2 of the 7 patients in our series there was no increase in the PBI^{131} level.

If this increase in PBI^{131} concentration were due largely to thyroglobulin one would expect an increase in BEI^{131} concentration as the thyroxine was released from metabolized thyroglobulin. Possibly such an increase occurred later. However, the BEI^{131} level might normally have decreased two days after the removal of most of the thyroid and the maintenance of a constant level might be due to the release of BEI^{131} from the PBI^{131}. In addition, some of the iodinated compounds released from thyroglobulin might have been metabolized within tissues without re-entering the circulation as BEI^{131}.

Although thyroidectomy had no immediate effect on the rate of disappearance of radiothyroxine in patients with treated Graves' disease, five to six days later there was a pronounced decrease in the rate. This was not associated with the discontinuance of drug therapy, since it occurred when the drugs were continued. It was not related to the time that had elapsed after the administration of radiothyroxine, as this varied from six days to eight days. In the present study, the slope of the values for plasma radioactivity was constant for eight days after administration of radiothyroxine (Fig. 1). Others (9, 11, 12) have also shown that there is no immediate change in this slope in the absence of thyroidectomy. If the initial observations in this study have been interpreted correctly as indicating that thyroid surgery releases iodinated protein from the thyroid, and if they can be applied to treated cases of Graves' disease as well as to normal subjects, then the addition of unlabeled thyroxine and its breakdown products to radiothyroxine might immediately slow the degradation rate of the radioactive thyroxine. Although the rate of turnover of radiothyroxine increases in proportion to the concentration of PBI (9), this is presumably due to chronic changes in the peripheral deiodinating systems. It has been shown (13) that, *in vitro*, the deiodinating system for radiothyroxine can be acutely saturated by added carrier-thyroxine. It also seems possible, on the basis of the findings in the present study, that thyroglobulin may un-

dergo proteolysis in the tissues and that the released stable thyroxine may compete advantageously with radiothyroxine for tissue binding sites at the place of deiodination.

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PITUITARY GIGANTISM IN A CHILD FIVE YEARS OF AGE: EFFECT OF X-RADIATION, ESTROGEN THERAPY AND SELF-IMPOSED STARVATION DIET DURING AN ELEVEN-YEAR PERIOD

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ABSTRACT

A case is presented of pituitary gigantism in a 5-year-old girl. The syndrome began at age 3, and was studied over a period of eleven years (from age 5 to age 16). X-radiation had a transitory lowering effect on the growth rate and on the level of serum inorganic phosphorus. Estrogen therapy also decreased the growth rate to some extent, but the retardation became pronounced with the onset of anorexia nervosa due to a self-imposed starvation diet.

PITUITARY gigantism is rarely diagnosed or even recognized in a patient 5 years of age. Such an opportunity arose through the observations of Dr. Jerome Chamovitz[1], who at the time was a Fellow in Internal Medicine at this clinic. He was impressed not only by the height of a child he had seen but also by the facial features, which suggested acromegaly. A few months later we were able to confirm his observations of abnormal stature and acromegalic appearance.

CASE REPORT

Patient R.C. was first seen in October 1947 when she was 5 years and 8 months old. Her mother stated that the patient was "just too big"; she weighed 70 pounds and was 50½ inches tall. The mother also reported that the girl wore a size 3 shoe, was awkward and clumsy, and stumbled frequently.

The child had been delivered by low forceps after version; at birth her length was 19 inches and weight 6.6 pounds.[2] She was bottle-fed until 3 months of age and thereafter drank from a glass. She crawled very early, but did not walk until the age of 16 months. She talked earlier than usual and used full sentences at age 2. No other abnormalities had been noted except that the child was very nervous. She had been in school only about three weeks.

At the time of her admission, a sister was 7½ years of age and was several inches taller but weighed the same. The father of the child was 6 feet tall and weighed 195 pounds.

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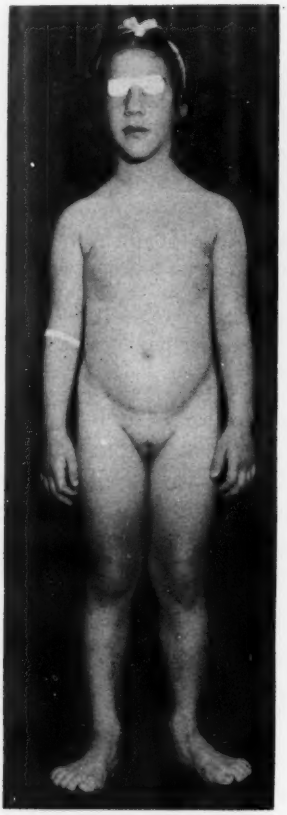
[1] Present address: Sewickley, Pennsylvania.

[2] We are indebted to the Boston Lying-In Hospital for this information.

The mother was about average height; her father was 5 feet 11 inches tall, and her mother 5 feet 1 inch. One of the mother's sisters was extremely obese.

The late Dr. Eli Romberg, pediatrician, recorded that at 7 weeks of age the child weighed $9\frac{1}{5}$ pounds and measured $22\frac{1}{4}$ inches in length. At $8\frac{1}{2}$ months she weighed 18 pounds, and at 11 months 19.8 pounds. The next observation was at 3 years of age, at which time the patient was $39\frac{1}{2}$ inches tall and weighed 37 pounds. Nothing unusual occurred between these examinations except for minor injuries and colds. At the time of



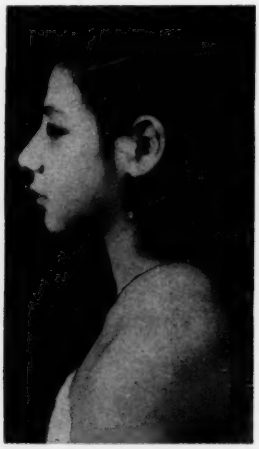


FIG. 1. (left) Patient at 5 years of age. Note prominent jaw despite lack of prognathism in lateral view (Fig. 2).

FIG. 2. (above) Lateral view of head and neck.

the last examination, Dr. Romberg expressed the opinion that the child was too large for her age.

Physical and laboratory examinations: When seen in 1947, the appearance strongly suggested mild acromegaly (Fig. 1); no prognathism was apparent in lateral view, however (Fig. 2). The child was alert and cooperative; she was left-handed in most activities although right-handed in eating. Until a few weeks before admission, she had practiced mirror writing when copying. As calculated by the Burgess table, the patient's stature of $50\frac{1}{2}$ inches corresponded to a height age of $8\frac{1}{2}$ years. The weight corresponded to that expected in a girl aged 10 or 11 years. Visceromegaly was not apparent. The blood pressure was 110 mm. Hg systolic and 70 mm. diastolic. A slight amount of pubic hair was

present; the exact date of its appearance was not known, but it was fairly recent. A roentgenogram of the skull showed that the sella measured 12 mm. by 12 mm., and the lateral area measured 102 sq. mm. The normal lateral area at 5 years of age is 48 sq. mm. (range 38 to 58), and at 10 years is 60 sq. mm. (range 45 to 75) (1). Dental age was 6 years. The sinuses seemed overdeveloped for the chronologic age (Fig. 3 *a*).

Roentgenograms of the hands (Fig. 4 *a*) showed normal bone texture with a slight tendency to tufting. Bone age was $5\frac{1}{2}$ to 6 years (Todd's standards).

The basal metabolic rate was +13 per cent. The hemoglobin level was 14.1 Gm. per 100 ml.; erythrocyte count 4,600,000 per cu. mm.; and differential count, 39.5 per cent

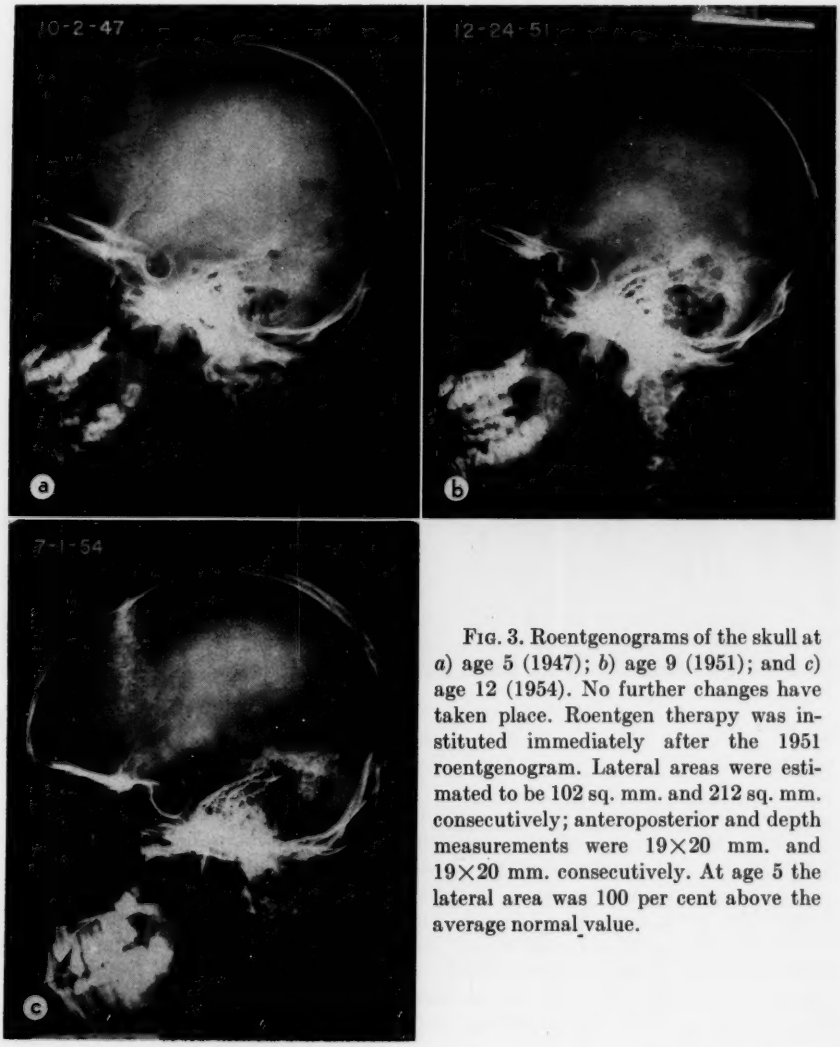


FIG. 3. Roentgenograms of the skull at *a*) age 5 (1947); *b*) age 9 (1951); and *c*) age 12 (1954). No further changes have taken place. Roentgen therapy was instituted immediately after the 1951 roentgenogram. Lateral areas were estimated to be 102 sq. mm. and 212 sq. mm. consecutively; anteroposterior and depth measurements were 19×20 mm. and 19×20 mm. consecutively. At age 5 the lateral area was 100 per cent above the average normal value.

polymorphonuclear neutrophils, 51 per cent lymphocytes, 8 per cent monocytes and 1 eosinophil. The nonfasting serum inorganic phosphorus level was 4.9 mg. per 100 ml. The concentration of blood sugar half an hour after eating was 84 mg. per 100 ml. The level of plasma cholesterol was 219 mg. per 100 ml.; of fasting serum phosphorus, 5.7 mg. per 100 ml.; and of alkaline phosphatase, 3.2 Bodansky units.

Treatment was postponed, chiefly because of parental objections. The patient was seen again in 1951 ($9\frac{1}{2}$ years old), at which time she was $61\frac{3}{4}$ inches tall—an increase of more than 10 inches in four years. She weighed 124 pounds. Some increase in pubic hair was noted.

Roentgenograms of the skull (Fig. 3 *b*) showed an increase in the size of the sella, the lateral area measuring 212 sq. mm. The cranial vault was somewhat thicker than in 1947. A roentgenogram of the wrist (Fig. 4 *b*) showed the epiphyses to be advanced to an age of approximately 10 years.

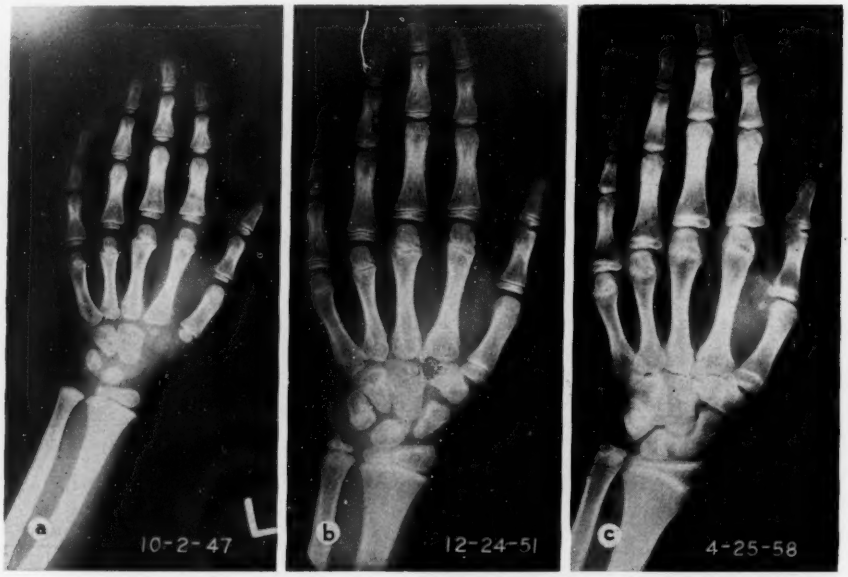


FIG. 4. Hand-wrist roentgenograms at *a*) age 5 (1947); *b*) age 9 (1951); and *c*) age 16 (1958). Bone ages were, respectively (Todd's atlas), 5 years 6 months, 10 years, and 13 years 9 months.

A few observations were made on a normal girl of approximately the same age (Fig. 5). The growth curve and serum phosphorus values are shown in Figure 6. Glucose and creatine tolerance curves are shown in Table 1 beside those of the patient.

X-ray therapy: It was decided to administer x-radiation to the pituitary, and a tumor dose of 2400 r was given by rotational supervoltage therapy, in a series of nine treatments. This treatment was completed on January 5, 1952. The fasting serum inorganic phosphorus level before treatment was 5.6 mg. per 100 ml.; March 22, 1952 it was 3.5 mg. per 100 ml. (The mean fasting concentration of serum inorganic phosphorus (2) at 9 years of age is approximately 4.5 mg. per 100 ml.) Height before treatment was $62\frac{1}{8}$–

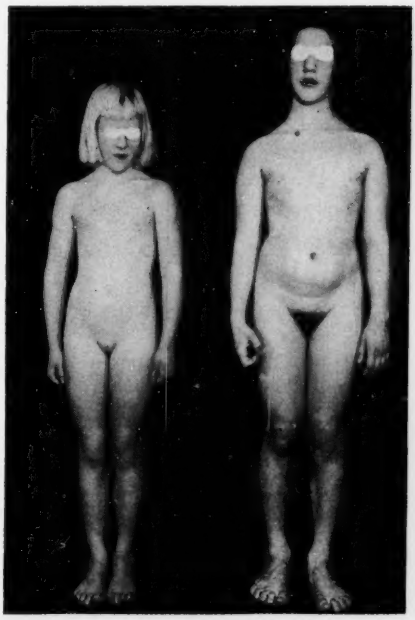


FIG. 5. Appearance of patient at 9 years 9 months of age (right) beside a normal girl, of 8 years 3 months (left). The patient's height on the Burgess chart at this time was well above the 99 percentile curve, indicating (according to Burgess) that only 1 in 10,000 girls would be of this height. The normal girl's height was 54.2 inches, which fell on the 99 percentile curve. Her growth has followed this curve to date.

62¼ inches; on March 22, 1952 it was 62¾ inches. On the basis of the projected curve, if the previous growth rate had continued, her height would have been 63¾ inches.

In May 1952, the serum phosphorus level had returned to 5.3 mg. per 100 ml. The patient had grown half an inch during the two preceding months, measuring 63¼ inches. This growth would be at the rate of 6 inches per year, whereas at most the rate should be 4 inches per year for her height (Burgess height chart). Serum phosphorus values on June 5, 1952 and June 13, 1952 were 4.7 mg. and 4.9 mg. per 100 ml., respectively.

Estrogen therapy: Since further x-ray treatment was not desired, stilbestrol (0.5 mg. daily) was started on September 3, 1952. Periodically, the child experienced vaginal bleeding, at which times the estrogen was discontinued temporarily. Administration of stilbestrol depressed the serum inorganic phosphorus level from 5.2 mg. to 4.3 mg. per

TABLE 1. GLUCOSE AND CREATINE TOLERANCE TESTS

December 1951

Time (hrs.)	Patient—Weight 122 pounds				Control—Weight 75 pounds				
	Blood sugar, (mg./100 ml.)	Inorganic phosphorus serum (mg./100 ml.)	Creatinine, serum (mg./100 ml.)	Creatine, urine (mg./100 ml.)	Potassium, serum (mEq./L.)	Blood sugar (mg./100 ml.)	Inorganic phosphorus, serum (mg./100 ml.)	Creatinine, serum (mg./100 ml.)	Creatine, urine (mg./100 ml.)
Fasting	85	5.3	2.6	13.4	4.6	85	4.5	2.2	17.8
½	98	5.7	2.7	16.1			4.7	2.6	20.2
1	83	5.3	2.7	20.8	4.8	78	4.7	5.7	14.8
2	93	5.0	6.1	13.9	4.2	66	4.4	6.4	21.6
3	84	5.0	5.7	15.0			5.1	6.4	21.6
	24-hour urine—vol. 665 ml. Creatinine, 1.8 Gm. Creatine, 0.5 Gm.				24-hour urine—vol. 800 ml. Creatinine, 0.8 Gm. Creatine, 0.1 Gm.				

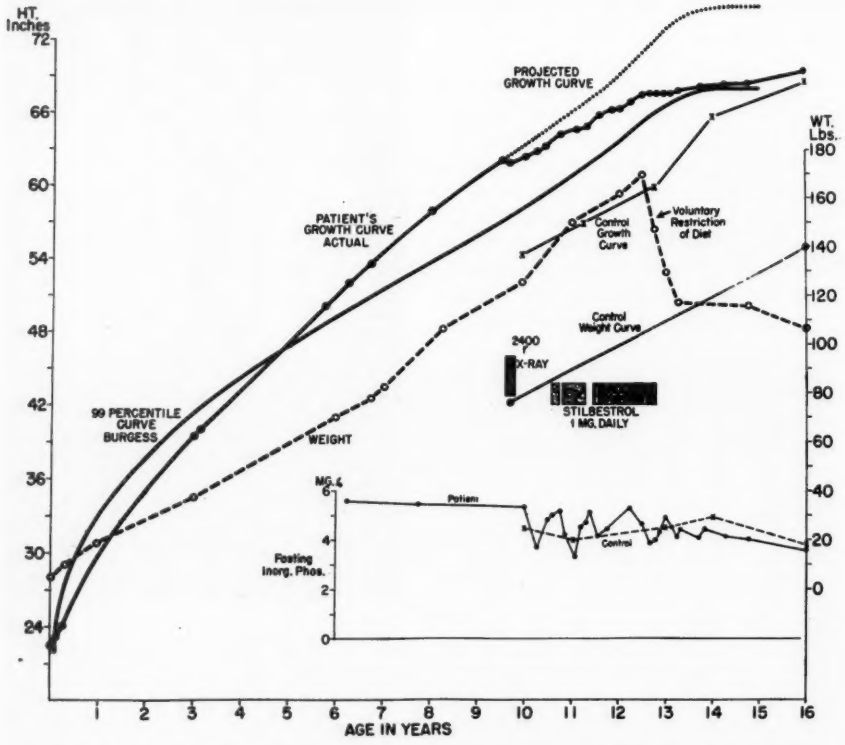


FIG. 6. Chart of patient's growth and treatment along with those for a tall normal girl (marked "control"). Note projected curve (dotted line) based on the assumption that growth would have continued at the same rate without treatment or onset of menarche. If growth had slowed spontaneously as the result of puberty, bone age should have accelerated, whereas at age 16 years the bone age was 13 years 9 months. Note prompt fall in the serum inorganic fasting phosphorus level and the return to previous values after roentgen therapy. Inorganic phosphorus values thereafter fluctuated during stilbestrol therapy. Of special interest is the decreased rate of growth following weight loss. (All measurements of height were made personally by the author.)

100 ml. in October 1952. In January 1953 it seemed that this therapy was as effective as the x-ray treatment in slowing her growth; the serum phosphorus level was 3.3 mg. per 100 ml., and height had increased only $\frac{2}{5}$ of an inch in three months. In July 1953 the patient had been taking stilbestrol continuously since April 28, 1953, and the serum inorganic phosphorus level was 4.1 mg. per 100 ml.

A year later (July 1954) the patient was taking 1 mg. of stilbestrol each day, and vaginal bleeding occurred about every two months. The serum inorganic phosphorus level was 4.7 mg. per 100 ml.; the patient weighed 170 pounds and was 67½ inches tall (Fig. 7). In September 1954 (while the patient was supposedly taking stilbestrol) the concentration of inorganic phosphorus was 4.0 mg. per 100 ml. In the light of subsequent developments, it is not certain how regularly the medication was taken.

Self-imposed starvation diet: At this time (July 1954) she apparently decided that she was too big, and did everything possible to avoid eating. It was discovered that the lunch which was prepared for her to take to school was discarded, and she resisted all attempts to increase her diet or continue medication. The only change noted was a slight increase in size of the thyroid gland, which suggested colloid accumulation. The vaginal smear showed a few round cells, but no glycogen (iodine vapor technique).

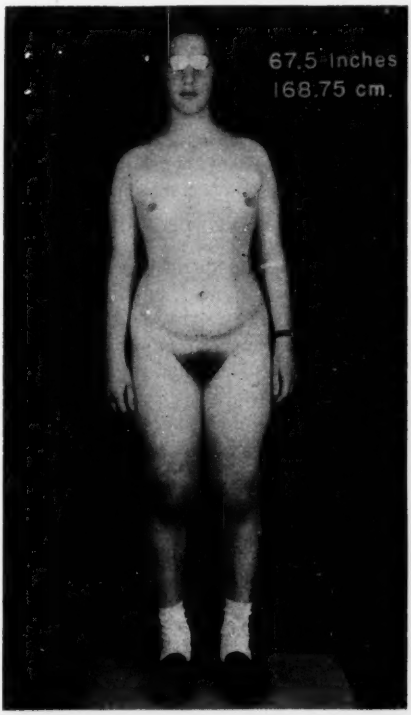


FIG. 7. Patient at 12 years of age. Note pigmentation of nipples and slight breast enlargement resulting from stilbestrol therapy.

By September 1954 she weighed 146 pounds and in July 1955 her weight was 120 pounds. Stilbestrol was not taken after October 1954. Nine of 10 determinations of the fasting serum phosphorus level from November 1954 to March 1958 were below 4.5 mg. per 100 ml. During this time she continued to lose weight, reaching 105 pounds. The final four phosphorus determinations were 4.1, 4.1, 4.0 and 3.7 mg. per 100 ml. On September 2, 1954, the serum calcium level was 10 mg. per 100 ml., and alkaline phosphate activity 5.0 Bodansky units; on December 29, 1955, alkaline phosphatase activity was 3.4 Bodansky units.

It should be noted that during the period of weight loss and thereafter, inorganic serum phosphorus values were normal or below, and the growth rate was much retarded. During this period, too, no estrin effect was observed in the vaginal smears on two occasions and there was no breast development.

The 24-hour excretion of 17-ketosteroids was as follows: at 5 years 8 months of age, 2.8 mg.; at 8 years 3 months, 5.1 mg.; at 9 years 9 months, 3.5 mg.; at 11 years, 8.8 mg.,

and at 16 years 3 months, 14.9 mg. Urinary corticoids at 16 years 3 months, were 12.5 mg. per twenty-four hours (normal 4.5 to 7.5 mg., Sulkowitch). A test for urinary estrogens in 1951 (age 9), yielded negative results.

The child had done very well in school, was overconscientious about her work, and tried for perfect grades in all subjects. When she entered junior high school, she could not keep up because of anxiety concerning her marks, and had to drop out.

She was then seen at the Children's Medical Center by Dr. Elliott H. Sweetser. Excerpts of his report are as follows: "The patient is extremely attached and dependent on her mother, eating only what her mother prepares for her. She is a perfectionist, and compulsive. She abhors the responsibility of being a grown-up woman, especially having menstruation. Our research group concurred in the diagnosis of anorexia nervosa. She has made some progress as regards nutrition. It seems that her attitude toward food is related to her intense and partly sadistic dependence on her mother. Perhaps in some unconscious way she knew how best to combat her gigantism."

Subsequently not much progress was made by psychiatric interviews, and they were accordingly discontinued. The patient maintained that she felt well, although in March 1958 she weighed only 105 pounds.

In 1958 she attended private school and it was hoped that she would be able to adjust to the new environment, but this attempt was also unsuccessful. When she was seen on April 25, 1958, her weight was 112 pounds, height $68\frac{5}{8}$ inches, bone age 13 years 9 months, and chronologic age 16. No further secondary sexual development was apparent. There was no anemia. The blood sugar (true glucose) level was 50 mg. per 100 ml. one and a half hours after meals; urinary 17-ketosteroids were 14.9 mg., and corticosteroids 12.5 mg. per twenty-four hours.

After the patient left school in the fall of 1958 she entered the Massachusetts Memorial Hospital under the care of Dr. Philip Kramer, who kindly sent us these observations. In his report he stated: "The patient is still a serious problem in feeding, although this is only part of her whole problem, which is one of an extremely disturbed individual. Her schemes to defeat our feeding are fantastic."

Endocrine studies at the Massachusetts Memorial Hospital yielded the following results: serum protein-bound iodine concentration, 4.8 μg. per 100 ml.; 24-hour urinary creatinine level, 0.69 mg. per 100 ml.; urinary 17-ketosteroids, 4.64 mg. per twenty-four hours; and no urinary gonadotropin (FSH) in dilutions 3.3 to 26.4 After administration of corticotropin gel (20 units twice a day for three days), the 24-hour urinary excretion of 17-ketosteroids was 19.9 mg., and of 11-oxysteroids 1.22 mg. The results of a five-hour oral glucose tolerance test, an intravenous glucose tolerance test and an insulin-glucose tolerance test were normal.

Final diagnoses at the M. M. Hospital were psychophysiologic gastro-intestinal disorder; obsessive-compulsive character; neurosis with depression; acromegaly and gigantism (arrested); pituitary eosinophilic adenoma.

Apparently no lasting improvement resulted from her hospitalization. At the present time the patient is in an institution for mental disease.

DISCUSSION

Acromegaly in classic form occurs after epiphyseal closure; widening of bones and prognathism are the characteristic features. Prognathism of a degree in which the lower jaw protrudes to the extent of overriding the lower front teeth beyond the upper teeth is most unusual in childhood.

Strictly defined, acromegaly means enlargement of the acral parts, including the face. We prefer to use the term "pituitary gigantism" to characterize hyperpituitarism beginning in childhood, and "acromegaly" to describe hyperpituitarism beginning after adolescence. Gigantism and prognathism which develop in the in-between age period justify the term "acromegalogigantism." Those who describe acromegaly in a child, however, are justified according to definition. Todd has recently reported such a case (3).

It is assumed that the cause of overgrowth in this patient was an eosinophilic adenoma. The sella was large for her age at 5 years (at least by 100 per cent above the mean). It is believed that the enlargement increased out of proportion to the growth rate, skull development and bone age. The accelerated growth rate was not caused by any tendency to sexual precocity with increased bone age, which is the usual finding in premature and often temporarily increased rate of growth. Pubic hair appeared early, and 17-ketosteroid excretion was virtually normal at ages 5, 8, 9, 11 and 16 years.

Roentgen therapy should have been carried out at the age of 5, but such treatment was deferred because of parental concern and some uncertainty on the part of the physician. When it was shown that the patient's growth curve indicated eventual gigantism, her parents consented to a trial of a little more than half the usual dose. The result of this was a slowing down of growth and a drop in the level of inorganic serum phosphorus to 3.5 mg. per 100 ml. the lowest value ever found in this case. This decrease was not permanent, however, and two months later the level had returned to 5.3 mg. per 100 ml. The use of fasting inorganic serum phosphorus values as an index of excessive growth hormone has been validated by Henneman (4) and by Raben (5). They observed a pronounced rise in the level of inorganic serum phosphorus (from 3.6 mg. to 6.0 mg. per 100 ml.) on administering growth hormone derived from human pituitary glands to pituitary dwarfs.

Before x-ray therapy the patient was growing at a rate of 3 inches a year and during the three to four months after x-ray therapy it was calculated that the growth rate was 1.9 inches per year. Stilbestrol was then tried, with a resultant lowering of the serum inorganic phosphorus concentration, which was less striking than that following x-ray therapy.

During the period of weight loss, from July 1954 to July 1955, the patient had grown only ¼ inch. It should be emphasized that height may decrease with weight loss, so that failure to increase in height temporarily may have been in part the result of this factor. Her subsequent growth in association with fairly constant weight indicates either a decrease in the production of growth hormone or its ineffectiveness in the presence of inadequate nutrition. The effect of malnutrition on growth has been

demonstrated (6). Bone age at this time was estimated to be from 13 years 3 months to 13 years 9 months (Todd's standards). No change in the size of the sella turcica or sinuses had occurred (Fig. 3 *c*).

The use of testosterone, with or without estrogen, to enhance epiphyseal closure was considered on several occasions. This was prescribed but the patient refused to take any medication. In another case of gigantism resulting from excessive growth hormone in a 15-year-old boy, epiphyseal closure was apparently the result of testosterone therapy because there had been no spontaneous sexual development to initiate it (7–9). It seems likely that the epiphyses cannot close without an increase in growth rate; this was one reason why large doses of testosterone were not administered. From January to November, 1953, while the patient was taking stilbestrol, there was an increase in bone age from 10.3 years to 12.9 years accompanied by a faster growth rate. This increase, however, could not be definitely related to stilbestrol therapy in view of the relative inaccuracies inherent in determining height and skeletal ages.

It also appears likely that, regardless of the amount of x-radiation administered (even to the point of inhibiting growth), sexual development and menarche would not have ensued, in view of the lack of urinary FSH together with clinical evidence of adequate thyroid and adrenal function. The malnutrition could have accounted for the absence of FSH as well as the cessation of excessive or normal secretion of growth hormone. A return to normal nutrition would probably have solved this problem, as it often does in cases of anorexia nervosa in adult women.

Addendum

Since this report was submitted the patient was seen again on February 5, 1961. There was no further sexual development or menstruation. Her weight was 195 pounds and height 70¼ inches—a gain of 1½ inches in thirty-three months. However, her height in December 1959 was recorded at 68½ inches (see below). The great increase in weight could have accounted for part of this gain in height. Dr. Thomas C. Hall of the McLean Hospital kindly supplied the following information: On admission, the patient weighed 85½ pounds and was 68½ inches tall. Recent investigations showed that urinary FSH was less than 6 mouse units, serum protein-bound iodine concentration 4.7 mg. per 100 ml., inorganic phosphorus 5.3 mg. per 100 ml., and alkaline phosphatase 5 units. Values for serum inorganic phosphorus up to 7.1 mg. per 100 ml. had been noted during 1960, and alkaline phosphatase values up to 11 units. The glucose tolerance curve was normal. Epiphyses of the hands were still open, although almost completely closed in the femora and tibiae. Certain questions arise: Did restoration of nutrition cause resumption of excess secretion of growth hormone, which in turn stimulated the voracious appetite? Will more signs of acromegaly develop, in view of the elevated levels of serum inorganic phosphorus?

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JCEM

The Endocrine Society

The 1961 Annual Meeting

The Forty-Third Annual Meeting of the Endocrine Society will be held in the Hotel Biltmore, New York, N. Y., Thursday, Friday and Saturday, *June 22, 23 and 24, 1961.*

The Scientific Sessions will be held from 9:00 a.m. to 5:00 p.m. daily and in addition there will be simultaneous afternoon sessions. The annual dinner is scheduled for Friday, June 23, at 7:30 p.m., preceded by cocktails at 6:30 p.m.

All members are urged to make their hotel reservations immediately. The Biltmore will hold 300 bedrooms for members until May 1, 1961, after which time the hotel will not guarantee further reservations. Therefore it is imperative that you make your reservations early, directly with the hotel, advising them of time and date of arrival and departure. If you plan on remaining through June 26 for the American Medical Association and other meetings, it is imperative that your reservations be made from June 26 on, directly through the A. M. A. Housing Bureau. Make your reservations early and avoid disappointment. Hotel reservation card will be mailed to members shortly after January 1, 1961.

Final program, membership card and advance registration forms will be sent on May 1, 1961 to members whose current dues have been paid.

The 1961 Awards

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors. Endocrine Society awards are limited to citizens of the United States and Canada.

Fred Conrad Koch Award

In 1957 a substantial legacy was bequeathed to the Society by the late Elizabeth Koch for the purpose of establishing the Fred Conrad Koch Memorial Fund in memory of her late husband, Distinguished Service

Professor of Physiological Chemistry at the University of Chicago and pioneer in the isolation of the androgens. This is the highest honor of The Endocrine Society and is represented by a medal known as the Koch Medal of The Endocrine Society. The medal, as well as an honorarium of $3,500, is to be given annually to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

This Award was first presented in 1959, to Dr. W. W. Swingle and in 1960 was presented to Dr. Emil Witschi. The Koch Medal has replaced the Medal of The Endocrine Society which was established in 1954.

The Ciba Award

The Ciba Award is given in recognition of the meritorious accomplishments of an investigator in the field of basic or clinical endocrinology who has not reached his thirty-sixth birthday before June 1 of the year in which the Award is presented. This Award was established in 1942 and first presented in 1944. The Award is presently in the amount of $2,500.

The Ayerst and the Squibb Fellowships

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

The Upjohn Scholar of the Endocrine Society

The Council of The Endocrine Society has established a category of Scholars. A grant has been made available through the generosity of the Upjohn Company, and will be awarded to an established investigator or teacher in the field of endocrinology who wishes to extend the opportunities for work either in this country or abroad.

The award will not exceed $2,500 annually and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Fred Conrad Koch Award, the Ciba Award, and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *October 15* each year.

Proposals for appointments as a Scholar of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *October 15* each year.

The American Goiter Association

The next Annual Meeting of the American Goiter Association will be held in the Warwick Hotel, Philadelphia, Pennsylvania, *May 3–6, 1961*. Arrangements are being made for a Clinical Day in the Philadelphia Hospitals on May 3. Scientific papers will be presented in the Warwick Hotel on May 4, 5 and 6. For further information address the Secretary, John C. McClintock, M.D., 702 Madison Avenue, Albany 8, N.Y.

Training Program in the Physiology of Reproduction

In recognition of the world-wide need for scientific training in reproductive physiology, the Worcester Foundation for Experimental Biology plans to continue the postdoctoral program in this field under a grant from the Population Council. Starting in 1962, the annual program will commence on February first.

Fellowships for the program will be awarded to candidates possessing the Ph.D. or M.D. degrees, or their equivalents. These Fellowships will carry a stipend of $5,500 per annum and will be for a twelve-month period. An allotment will also be made for round-trip travel to Shrewsbury, Massachusetts. Application forms may be secured from the Program Director of the course in the Physiology of Reproduction, and should be returned *not later than June first.* They will be processed by an advisory and selection committee comprised of experts, and the Fellowship awards will be announced by September 15.

Individuals interested in participating in the program may communicate with Doctor Celso-Ramon Garcia, Program Director, at any time. However, applications will not be acted upon prior to the given date for the particular year selected. Whenever possible, arrangements should be made for an interview with a member of the selection committee. The members of the advisory and selection committee are:

Doctor Gregory Pincus,
Worcester Foundation for Experimental Biology,
Shrewsbury, Massachusetts

Doctor Warren O. Nelson,
The Population Council, Inc.,
Rockefeller Institute
66th Street & York Avenue,
New York 21, N. Y.

Doctor V. R. Khanolkar,
Indian Cancer Research Centre,
Parel, Bombay 12, India

Doctor Roberto Caldeyro-Barcia,
Servicio de Fisiologia Obstetrica,
Facultad de Medicine,
Avenida Gral. Flores, 2125,
Montevideo, Uruguay

Doctor Takeshi Nakao,
Tokyo Jikei-kai School of Medicine,
Department of Pharmacology,
Atago-cho, Shiba, Minato-ku,
Tokyo, Japan

Doctor M. C. Shelesnyak,
Weizmann Institute of Science,
P. O. Box 26,
Rehovoth, Israel.

The program will consist of a course of lectures and laboratory work. During the latter part of the year, each Fellow will have the opportunity to participate in a research project pertinent to his interests and within the capacity of the program.

The lecture series will be given by the Program Director, various members of the staff of the Worcester Foundation, and guest experts. The lectures and accompanying laboratory work will cover various phases of reproductive processes, particularly in mammals. Subjects to be treated will involve a review of reproductive processes in the male, including considera-

tion of spermatogenesis hormonogenesis, the control of testis function, and the functional endocrinology of testis hormones. Extensive attention will be given to oogenesis, ovulation, and fertilization in the female, factors affecting the entire course of pregnancy and embryonic development, as well as the postnatal physiology of the female. Additional topics will include the role of extra-reproductive tract organs in reproductive processes, genetic and nutritive factors in reproduction, and the control of fertility and sterility.

Laboratory exercises will include the study of characteristic reproductive phenomena in various species, analytic procedures employed in determining the chemistry of reproductive organs, the biochemistry of spermatozoa and ova, and hormone analyses pertinent to gonad function, to sex cycles, to gestation and lactation.

Postgraduate Course—Laboratory Tests in Diagnosis and Investigation of Endocrine Functions

This course will be given in Toland Hall, University of California Medical Center, Third and Parnassus, San Francisco, California, *April 7–9, 1961*. Thirty-five leading investigators will cover, among other things, the present findings in the field of endocrinology that show some promise for clinical use in the future. There will be seven sessions devoted to the pituitary, thyroid, parathyroids, pancreatic islets, adrenals, testes, and ovaries, in that order. The fee is $50.00 (dinner, $5.00). For additional information, write to the Program Chairman, Dr. Roberto F. Escamilla, Clinical Professor of Medicine, University of California Medical Center, San Francisco 22, California.

Postgraduate Courses in Pediatrics—Harvard Medical School

1. Pediatrics—*June 5–16 inclusive, 1961;* daily 9:15 a.m., to 4:30 p.m.' Massachusetts General Hospital. This course provides a general review with emphasis on recent advances and therapy. The first seven days are devoted to pediatric medical, surgical, urologic, orthopedic, neurologic, cardiac, allergic, dermatologic, psychiatric, eye, ear, and x-ray problems. The last four days concentrate on disorders of growth and development, common endocrine problems, and the metabolic aspects of infant feeding, parenteral fluid therapy and management of nephritic and diabetic patients.

2. Pediatric Metabolism and Endocrinology—*June 13–16 inclusive, 1961;* daily 9:15 a.m. to 4:30 p.m. Massachusetts General Hospital. This course is the same as the last four days of the course in Pediatrics, but may be taken separately.

Those interested should write: Courses for Graduates, Harvard Medical School, 25 Shattuck Street, Boston, Massachusetts.

International Symposium on Host Tumor Interactions

An international symposium with the title "Host Tumor Interactions" has been scheduled for *May 18–20, 1961*, at the Henry Ford Hospital in Detroit, Michigan. The symposium will deal with the mechanisms of cell and tissue interactions at a basic biological level and attempt to provide an interpretation of certain aspects of neoplastic disease on this foundation. Attendance at the symposium is limited to 400 invited speakers and guests. Further information may be obtained from Michael J. Brennan, M.D., Oncology Division, Secretary, Symposium on Host Tumor Interactions, Henry Ford Hospital, Detroit 2, Michigan.

Book Register

Experimental Study on the Metabolism of some Synthetic Corticoids and on the Influence of their Therapeutic Use on Adrenal Cortical Function. (Includes English summary of foreign language text.) By DR. A. VERMEULEN, Rijksuniversiteit te Gent (Director, Prof. Dr. P. Regniers). Synthetic and natural corticosteroids are compared in two studies concerning 1) their metabolic transformations, and 2) their effect on pituitary-adrenal function. A decreased inactivation rate and reduced protein-binding partly account for the increased glucocorticoid activity of prednisolone, prednisone, triamcinolone and dexamethasone. Adrenal hypofunction after corticoid withdrawal is frequent and persistent; paper; 236 pages; 1960. Arsica Uitgaven N. V., Stoofstraat 60, Brussels, Belgium.

Haemopoiesis. Cell Production and Its Regulation. Ciba Foundation Symposium edited by G. E. W. WOLSTENHOLME, M.B., M.R.C.P. and M. O'CONNOR, B.A. The Symposium was held February 2–4, 1960, and the participants discussed such subjects as: Use of Tritiated Thymidine in the Study of Haemopoietic Cell Proliferation; Differentiation, Proliferation and Maturation of Haemopoietic Cells Studied in Tissue Culture; Destination of Thymus Lymphocytes; Production and Distribution of Granulocytes and the Control of Granulocyte Release; Mobilization of Vitamin B_{12} in Response to Acute Blood Loss; Humoral Influences on Blood Cell Formation and Release; Factors in the Control of Haemopoiesis; Sources and Properties of Human Urinary Erythropoietin; Transfusion-Induced Polycythaemia as a Model for Studying Factors Influencing Erythropoiesis; 490 pages; 107 illustrations; 1960. Little, Brown & Company, Boston, Massachusetts. Price $11.00.

Internationales Biguanid-Symposium. Edited by PROF. DR. F. BERTRAM, Hamburg, and DR. G. MICHAEL, Stolberg (Rhld.). (In German.) The Symposium was held May 12 and 13, 1960, in Aachen. Various investigators in the field present papers on the chemistry and pharmacology of the biguanides, their toxicity and renal action, and experiences with biguanides in the United States and Canada. The last section deals with "Klinik der Biguanide" in 11 presentations covering the use of biguanides in the therapy of various forms of diabetes. Discussions are given a prominent place; paper; 167 pages; 43 illustrations; 1960. Georg Thieme Verlag, Stuttgart. In the U.S.A. and Canada, Intercontinental Medical Book Corporation, New York 16, N. Y. Price, DM 16.80; $4.00.

L'Année endocrinologique. Twelfth year. By M. ALBEAUX-FERNET, L. BELLOT, P. BUGARD, L. CANET, J. CHABOT, J. DERIBREUX, M. GELINET and J.-D. ROMANI. (In French.) This yearly volume contains articles dealing with the newer knowledge of the pituitary-adrenal axis, pheochromocytoma, nontumoral hirsutism, the adrenal cortex in catabolic states, the distribution of radioactive iodine, hyperthyroidism in the adult, thyroiditis auto-antibodies, triiodothyronine and the sex hormones, human somatotropin, various hypophyseal syndromes, the results of hypophysectomy, intersexuality, practical tests of ovulation, the remaining ovary after unilateral ovariectomy, biguanides in the treatment of diabetes, corticosteroid therapy in neurovascular lesions, and the treatment of the Waterhouse-Friderichsen syndrome; paper; 210 pages; 1960. Masson et Cie, Paris 6, France.

Man and His Body. The Wonders of the Human Mechanism. By BENJAMIN F. MILLER, M.D., Director of the May Institute for Medical Research of the Cincinnati Jewish Hospital and Associate Professor at the University of Cincinnati College of Medicine, and RUTH GOODE, senior staff writer of the magazine *MD.* The complex and efficient mechanism of the body is discussed in 22 chapters, including such subjects as The Body in Motion, The Rivers of Life, The Chemical Workshop, Ordinary Star-Stuff, A Billion Quiet Fires, Cycles of Growth, Biological Love Story, Mind and Mechanisms, The Unique You, and Man of the Future; 375 pages; illustrated; 1960. Simon and Schuster, New York 20, N. Y. Price $5.95.

Radioisotope Measurement in Nuclear Medicine. By FRANK H. LOW. One of a series of technical bulletins published as a service to the medical profession, written in easily understood terms and illustrated with many drawings. The Appendix contains radioisotope data, formulas and charts on radiation shielding, counting statistics, source activity and clinical calculations, and information on resolving time; 67 pages; 1960. Picker X-Ray Corporation, 75 Varick Street, New York, N. Y. Price $2.50.